

COMPUTER SYSTEMS AND METHODS FOR CONSTRUCTING BIOLOGICAL CLASSIFIERS AND USES THEREOF

This application is entitled to and claims priority benefit under 35 U.S.C. Section 119(e) to U.S. provisional application Number 60/581,312, filed June 19, 2004, U.S. provisional application Number 60/581,977, filed June 21, 2004, U.S. provisional application Number 60/643,475, filed January 12, 2005, and U.S. provisional application Number 60/663,722, filed March 22, 2005, each of which is incorporated herein by reference in its entirety.

1. FIELD OF THE INVENTION

The field of this invention relates to computer systems and methods to identify classifiers using data obtained from blood. The invention further encompasses the use of the classifiers and combinations of molecular markers identified by the classifiers in a wide variety of applications including: diagnosis; prognosis; prediction of disease, stage of disease or disease risk; monitoring disease progression and/or regression; monitoring disease reoccurrence and identifying risk of disease reoccurrence; determining and/or predicting response to treatment and/or treatment outcomes; monitoring and/or predicting treatment compliance or non compliance and the like.

<u>Table</u>	<u>DESCRIPTION</u>	<u>SIZE</u>	<u>Date Recorded</u>	<u>Text File Name</u>
IA	Sequence Related Table regarding Comorbid Hypertension	96KB	June 17, 2005	TABLE1A.TXT
IB	Sequence Related Table regarding Comorbid Obesity	102KB	June 17, 2005	TABLE1B.TXT
1C	Sequence Related Table regarding Comorbid Allergies	49KB	June 17, 2005	TABLE1C.TXT
ID	Sequence Related Table regarding Comorbid Systemic Steroids	59KB	June 17, 2005	TABLE1D.TXT
IE	Sequence Related Table regarding Hypertension (Chondro)	204KB	June 17, 2005	TABLE1E.TXT
IF	Sequence Related Table regarding Obesity (Chondro)	251KB	June 17, 2005	TABLE1F.TXT
IG	Sequence Related Table regarding CoMorbid Hypertension Only	57KB	June 17, 2005	TABLE1G.TXT
IH	Sequence Related Table regarding Hypertension OA Shared	23KB	June 17, 2005	TABLE1H.TXT
II	Sequence Related Table regarding Comorbid Obesity Only	60KB	June 17, 2005	TABLE1I.TXT

IJ	Sequence Related Table regarding Obesity OA Shared	18KB	June 17, 2005	TABLE1J.TXT
IK	Sequence Related Table regarding Comorbid Allergy Only	28KB	June 17, 2005	TABLE1K.TXT
IL	Sequence Related Table regarding Allergy OA Shared	19KB	June 17, 2005	TABLE1L.TXT
IM	Sequence Related Table regarding Comorbid Steroid Shared	40KB	June 17, 2005	TABLE1M.TXT
IN	Sequence Related Table regarding Steroid OA Shared	23KB	June 17, 2005	TABLE1N.TXT
IO	Sequence Related Table regarding Differentiating Systemic Steroids (49KB	June 17, 2005	TABLE1O.TXT
IP	Sequence Related Table regarding Diabetes	118KB	June 17, 2005	TABLE1P.TXT
IQ	Sequence Related Table regarding Hyperlipidemia	165KB	June 17, 2005	TABLE1Q.TXT
IR	Sequence Related Table regarding Lung Disease	102KB	June 17, 2005	TABLE1R.TXT
IS	Sequence Related Table regarding Bladder Cancer	830KB	June 17, 2005	TABLE1S.TXT
IT	Sequence Related Table regarding Bladder Cancer Staging	483KB	June 17, 2005	TABLE1T.TXT
IU	Sequence Related Table regarding Coronary Artery Disease	657KB	June 17, 2005	TABLE1U.TXT
IV	Sequence Related Table regarding Rheumatoid Arthritis	380KB	June 17, 2005	TABLE1V.TXT
IW	Sequence Related Table regarding Rheumatoid Arthritis	183KB	June 17, 2005	TABLE1W.TXT
IX	Sequence Related Table regarding Depression	165KB	June 17, 2005	TABLE1X.TXT
IY	Sequence Related Table regarding OAStaging	32KB	June 17, 2005	TABLE1Y.TXT
IZ	Sequence Related Table regarding Liver Cancer	19KB	June 17, 2005	TABLE1Z.TXT
IZb	Sequence Related Table regarding Liver Cancer	430KB	June 17, 2005	TABLE1Z.TXT
IAA	Sequence Related Table regarding Schizophrenia	592KB	June 17, 2005	TABLE1AA.TXT
IAB	Sequence Related Table regarding Chagas Disease	142KB	June 17, 2005	TABLE1AB.TXT
IAC	Sequence Related Table regarding Asthma (Chondro)	64KB	June 17, 2005	TABLE1AC.TXT
IAD	Sequence Related Table regarding Asthma (Affy)	57KB	June 17, 2005	TABLE1AD.TXT

IAE	Sequence Related Table regarding Lung Cancer	118KB	June 17, 2005	TABLE 1AE.TXT
IAG	Sequence Related Table regarding Hypertension (Afrymetrix)	157KB	June 17, 2005	TABLE1AG.TXT
IAH	Sequence Related Table regarding Obesity (Affymetrix)	203KB	June 17, 2005	TABLE1AH.TXT
IAI	Sequence Related Table regarding Ankylosing Spondylitis (Afify)	267KB	June 17, 2005	TABLE1AI.TXT
2	Sequence Related Table regarding OA Only Subtraction	19KB	June 17, 2005	TABLE2.TXT
3A	Sequence Related Table regarding Schizophrenia v. MDS	228KB	June 17, 2005	TABLE3A.TXT
3B	Sequence Related Table regarding Hepatitis v. Liver Cancer	347KB	June 17, 2005	TABLE3B.TXT
3C	Sequence Related Table regarding Bladder Cancer v. Kidney Cancer	470KB	June 17, 2005	TABLE3C.TXT
3D	Sequence Related Table regarding Bladder Cancer v. Testicular Cancer	556KB	June 17, 2005	TABLE3D.TXT
3E	Sequence Related Table regarding Testicular Cancer v. Kidney Cancer	588KB	June 17, 2005	TABLE3E.TXT
3F	Sequence Related Table regarding Liver Cancer v. Stomach Cancer	84KB	June 17, 2005	TABLE3F.TXT
3G	Sequence Related Table regarding Liver Cancer v. Colon Cancer	149KB	June 17, 2005	TABLE3G.TXT
3H	Sequence Related Table regarding Stomach Cancer v. Colon Cancer	166KB	June 17, 2005	TABLE3H.TXT
3I	Sequence Related Table regarding OA v. RA	214KB	June 17, 2005	TABLE3I.TXT
3K	Sequence Related Table regarding Chagas Disease v.Heart Failure	16KB	June 17, 2005	TABLE3K.TXT
3L	Sequence Related Table regarding Chagas Disease v. CAD	19KB	June 17, 2005	TABLE3L.TXT
3N	Sequence Related Table regarding CAD v. Heart Failure	13KB	June 17, 2005	TABLE3N.TXT
3P	Sequence Related Table regarding Asymptomatic Chagas v. Symptomatic	68KB	June 17, 2005	TABLE3P.TXT

	Chagas			
3Q	Sequence Related Table regarding Alzheimers' v. Schizophrenia	56KB	June 17, 2005	TABLE3Q.TXT
3R	Sequence Related Table regarding Alzheimers' v. Manic Depression	51KB	June 17, 2005	TABLE3R.TXT
4A	Sequence Related Table regarding OA v. Control (ChondroChip)	538KB	June 17, 2005	TABLE4A.TXT
4B	Sequence Related Table regarding OA v. Control (Affy)	550KB	June 17, 2005	TABLE4B.TXT
4C	Sequence Related Table regarding OA mild v. Control (ChondroChip)	321KB	June 17, 2005	TABLE4C.TXT
4D	Sequence Related Table regarding OA mild v. Control (Affy)	587KB	June 17, 2005	TABLE 4D.TXT
4E	Sequence Related Table regarding OA moderate v. Control (ChondroChip)	198KB	June 17, 2005	TABLE4E.TXT
4F	Sequence Related Table regarding OA moderate v. Control (Affy)	576KB	June 17, 2005	TABLE4F.TXT
4G	Sequence Related Table regarding OA marked v. Control (ChondroChip)	203KB	June 17, 2005	TABLE4G.TXT
4H	Sequence Related Table regarding OA marked v. Control (Affy)	679KB	June 17, 2005	TABLE4H.TXT
4I	Sequence Related Table regarding OA severe v. Control (ChondroChip)	291KB	June 17, 2005	TABLE4I.TXT
4J	Sequence Related Table regarding OA severe v. Control (Affy)	607KB	June 17, 2005	TABLE4J.TXT
4K	Sequence Related Table regarding OA mild v. moderate (ChondroChip)	113KB	June 17, 2005	TABLE4K.TXT
4L	Sequence Related Table regarding OA mild v. moderate (Affy)	488KB	June 17, 2005	TABLE4L.TXT
4M	Sequence Related Table regarding OA mild v. marked (ChondroChip)	93KB	June 17, 2005	TABLE4M.TXT
4N	Sequence Related Table regarding OA mild v. marked (Affy)	373KB	June 17, 2005	TABLE4N.TXT
4O	Sequence Related Table regarding OA mild v. severe	177KB	June 17, 2005	TABLE4O.TXT

	(ChondroChip)			
4P	Sequence Related Table regarding OA mild v. severe (Affy)	687KB	June 17, 2005	TABLE4P.TXT
4Q	Sequence Related Table regarding OA moderate v. marked (ChondroChip)	103KB	June 17, 2005	TABLE4Q.TXT
4R	Sequence Related Table regarding OA moderate v. marked (Affy)	450KB	June 17, 2005	TABLE4R.TXT
4S	Sequence Related Table regarding OA moderate v. severe (ChondroChip)	79KB	June 17, 2005	TABLE4S.TXT
4T	Sequence Related Table regarding OA moderate v. severe (Affy)	627KB	June 17, 2005	TABLE4T.TXT
4U	Sequence Related Table regarding OA marked v. severe (ChondroChip)	66KB	June 17, 2005	TABLE4U.TXT
4V	Sequence Related Table regarding OA marked v. severe (Affy)	758KB	June 17, 2005	TABLE4V.TXT
5A	Sequence Related Table regarding Psoriasis v. Control	80KB	June 17, 2005	TABLE5A.TXT
5B	Sequence Related Table regarding Thyroid Disorder v. Control	373KB	June 17, 2005	TABLE5B.TXT
5C	Sequence Related Table regarding Irritable Bowel Syndrome v. Control	87KB	June 17, 2005	TABLE5C.TXT
5D	Sequence Related Table regarding Osteoporosis v. Control	79KB	June 17, 2005	TABLE5D.TXT
5E	Sequence Related Table regarding Migraine Headaches v. Control	231KB	June 17, 2005	TABLE5E.TXT
5F	Sequence Related Table regarding Eczema v. Control	56KB	June 17, 2005	TABLE5F.TXT
5G	Sequence Related Table regarding NASH v. Control	349KB	June 17, 2005	TABLE5G.TXT
5H	Sequence Related Table regarding Alzheimers' v. Control	268KB	June 17, 2005	TABLE5H.TXT
5I	Sequence Related Table regarding Manic Depression v. Control	298KB	June 17, 2005	TABLE5I.TXT
5J	Sequence Related Table regarding Crohns' Colitis v. Control	45KB	June 17, 2005	TABLE5J.TXT
5K	Sequence Related Table regarding Chronic Cholecystitis	53KB	June 17, 2005	TABLE5K.TXT

	v. Control			
5L	Sequence Related Table regarding Heart Failure v. Control	160KB	June 17, 2005	TABLE5L.TXT
5M	Sequence Related Table regarding Cervical Cancer v. Control	304KB	June 17, 2005	TABLE5M.TXT
5N	Sequence Related Table regarding Stomach Cancer v. Control	185KB	June 17, 2005	TABLE5N.TXT
5O	Sequence Related Table regarding Kidney Cancer v. Control	404KB	June 17, 2005	TABLE5O.TXT
5P	Sequence Related Table regarding Testicular Cancer v. Control	486KB	June 17, 2005	TABLE5P.TXT
5Q	Sequence Related Table regarding Colon Cancer v. Control	380KB	June 17, 2005	TABLE5Q.TXT
5R	Sequence Related Table regarding Hepatitis B v. Control	140KB	June 17, 2005	TABLE5R.TXT
5S	Sequence Related Table regarding Pancreatic Cancer v. Control	177KB	June 17, 2005	TABLE5S.TXT
5T	Sequence Related Table regarding Asymptomatic Chagas v. Control	63KB	June 17, 2005	TABLE5T.TXT
5U	Sequence Related Table regarding Symptomatic Chagas v. Control	77KB	June 17, 2005	TABLE5U.TXT
5V	Sequence Related Table regarding Bladder Cancer v. Control	383KB	June 17, 2005	TABLE5V.TXT
6A	Sequence Related Table regarding Cancer (all types) v. Control	163KB	June 17, 2005	TABLE6A.TXT
6B	Sequence Related Table regarding Cardiovascular Disease v. Control	73KB	June 17, 2005	TABLE6B.TXT
βc	Sequence Related Table regarding Neurological Diseases v. Control	337KB	June 17, 2005	TABLE6C.TXT
7A	Sequence Related Table regarding Celebrex® v. all Cox inhibitors except Celebrex	55KB	June 17, 2005	TABLE7A.TXT
7B	Sequence Related Table regarding Celebrex® v. Control	57KB	June 17, 2005	TABLE7B.TXT
7C	Sequence Related Table	53KB	June 17,	TABLE7C.TXT

	regarding Vioxx® v. Control		2005	
7D	Sequence Related Table regarding Vioxx® v. All Cox Inhibitors except Vioxx®	49KB	June 17, 2005	TABLE7D.TXT
7E	Sequence Related Table regarding NSAIDS v. Control	72KB	June 17, 2005	TABLE7E.TXT
7F	Sequence Related Table regarding Cortisone v. Control	208KB	June 17, 2005	TABLE7F.TXT
7G	Sequence Related Table regarding Visco Supplement v. Control	316KB	June 17, 2005	TABLE7G.TXT
7H	Sequence Related Table regarding Lipitor® v. Control	131KB	June 17, 2005	TABLE7H.TXT
7I	Sequence Related Table regarding Smoker v. Non- Smoker	23KB	June 17, 2005	TABLE7I.TXT

2. BACKGROUND OF THE INVENTION

The prior art is deficient in simple, non-invasive and effective methods of identifying molecular markers and the use of said molecular markers for purposes of: diagnosis; prognosis; prediction of disease, stage of disease or disease risk; monitor

5 disease progression and/or regression; monitor disease reoccurrence and identifying risk of disease reoccurrence or the like. The prior art is also deficient in simple non-invasive methods of identifying molecular markers and use of said molecular markers to determine and/or predict response to treatment and/or treatment outcomes, monitor and/or predict treatment compliance or non-compliance, etc. Although progress has been made in

10 identifying molecular markers by detecting the products of putative molecular markers using expression arrays in a variety of diagnostic areas and therapeutic areas, such progress has been primarily limited to studying non-blood tissue samples, such as primary tumors, that are difficult to obtain and thus have limited potential as a diagnostic. What is even more unsatisfactory is that retrieval of such tissue samples often requires invasive

15 medical procedures such as surgery. Prediction of response to treatment is also a significant problem. It is well understood that, for many currently recognized treatments, only a small percentage of the population (for example approximately 20-30%) will respond positively. Amongst the remainder of the population, there are those who do not improve, and others who display a negative or toxic response to the treatment. As a result

20 of these detrimental effects to some, many effective treatments do not get to market. The prior art is thus deficient in simple, non-invasive methods to analyze and predict treatment and response to treatment.

Such drawbacks have made identification of molecular markers unsatisfactorily difficult. See, for example, Alon *et al*, 1999, Proc. Natl. Acad. Sci. USA 96, pp. 6745-6750; Schummer *et al*, 1999, Gene 238, pp. 375-385; and van't Veer, 2002, Nature 415, pp. 530-536.

5 Even where progress has been made in identifying molecular markers by monitoring molecular marker products using expression arrays - whether in blood or using tissue - the techniques utilized merely identify large number of molecular markers two or more of which may be required so as to permit categorizing an unknown sample for diagnosis. It is not clear, however, which of these molecular markers are most useful to
10 accurately diagnose an unknown sample. In addition, techniques currently available in the art are not sufficiently robust (ie high levels of reproducibility) in accordance with scientific and regulatory standards so as to be used reliably to diagnose a test individual. Thus what is required in the art is a means to select smaller subsets of useful molecular markers which when used in combination permit the accurate and reproducible diagnosis
15 of an unknown sample for a particular trait of interest. Further what is required in the art is a means of translating the molecular marker data from these selected combinations so as to convert these into a diagnosis.

Discussion or citation of a reference herein will not be construed as an admission that such reference is prior art to the present invention.

20 3. SUMMARY OF THE INVENTION

Thus what is needed in the art is a method to identify useful combinations of molecular markers and a means of using said combinations of molecular markers (or more accurately measurement of the products of said molecular markers) so as to permit diagnosis of a test sample. Embodiments of the present invention address many of the
25 shortcomings and drawbacks found in the prior art by the novel approach of using molecular marker measurement data from blood and methods of processing such data to screen the large numbers of candidate molecular markers in blood so as to identify useful combinations of these molecular markers. Embodiments of the present invention involve the construction of classifiers and use of these classifiers. In addition, embodiments of the
30 invention involve the use of the molecular markers identified by these classifiers to diagnose or otherwise determine whether a test subject has a specific trait of interest. Blood offers a surprisingly informative alternative to tissues as a source of information. Blood includes numerous cell types including monocytes, leukocytes, lymphocytes, erythrocytes, platelets, as well as possibly many other cell types. The turnover of cells in

the human circulatory system is rapid. As a consequence of continuous interactions between the blood and the body, it has been hypothesized that the changes that occur within the cells or tissues of the body will trigger specific changes in gene expression within blood. See, for example, United States patent application serial No. 10/601,518, filed June 20, 2003, United States patent application serial No. 10/802,875, filed March 12, 2004, United States patent application serial No. 10/809,675, filed March 25, 2004, United States patent application serial No. 10/268,730, filed October 9, 2002, United States patent application serial No. 09/477,148, filed January 4, 2000, and United States patent application serial No. 60/115,125, filed Jan. 6, 1999, which are hereby incorporated herein by reference in their entirety. Thus, blood has the potential to provide a powerful indicator of what is happening in the human body at any given time, but provides unique challenges to harness this vast amount of potential information available. Embodiments of the current invention help address this challenge.

4. BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 illustrates a computer system for determining and selecting useful biological classifiers.

Fig. 2 illustrates a method for deriving biological classifiers in accordance with an embodiment of the present invention.

Fig. 3 is a flowchart of a method of applying the classifiers to a patient.

Fig. 4 illustrates a data structure for storing high throughput information for a plurality of molecular markers in accordance with one embodiment of the present invention.

Fig. 5 illustrates a data structure for storing a plurality of classifiers in accordance with one embodiment of the present invention.

Fig. 6 illustrates a patient database for storing data for molecular markers for a plurality of patients in accordance with an embodiment of the present invention.

Fig. 7 illustrates a Receiver Operating Characteristic (ROC) curve that is used to assess the discriminating ability of a molecular marker or a classifier in accordance with one embodiment of the present invention.

Fig. 8 illustrates ROC curves corresponding to two candidate classifiers for osteoarthritis computed in accordance with one embodiment of the present invention.

Description of Tables;

Table 1 as a group of tables identifies the molecular markers that are differentially expressed in blood samples from patients with a disease or patients who are co-morbid as

compared to blood samples from healthy patients or patients without said disease, or with only one of said co-morbid diseases and also shows the sequences of selected products of the identified molecular markers. Molecular marker data from the molecular markers listed in each table or a subset of these molecular markers can be used in steps 214-
5 218 as outlined in Figure 2B so as to identify classifiers and the combinations of molecular markers which form the classifiers useful in diagnosis.

Table 1A identifies the molecular markers which are differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member of the subgroup has both osteoarthritis and hypertension as compared with a
10 second trait subgroup wherein each member of the second trait subgroup has neither osteoarthritis nor hypertension using the ChondroChip™ platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 1B shows the identity of those molecular markers that are differentially expressed in blood samples from a training population comprised of a first trait subgroup
15 where each member of the subgroup has both osteoarthritis and obesity as compared with a second trait subgroup wherein each member of the second trait subgroup has neither osteoarthritis nor obesity using the ChondroChip™ platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 1C shows the molecular markers that are differentially expressed in blood
20 samples from a training population comprised of a first trait subgroup where each member of the subgroup has both osteoarthritis and allergies as compared with a second trait subgroup wherein each member of the second trait subgroup has neither osteoarthritis nor allergies using the ChondroChip™ platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 1D shows the molecular markers that are differentially expressed in blood
25 samples from a training population comprised of a first trait subgroup where each member of the subgroup has both osteoarthritis and subject to systemic steroids as compared with normal patients using the ChondroChip™ platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 1E shows the molecular markers that are differentially expressed in blood
30 samples from a training population comprised of a first trait subgroup where each member of the subgroup has hypertension as compared to a second trait subgroup wherein each member of the second trait subgroup did not have hypertension using the ChondroChip™

platform. The table also shows the sequences of selected products of the identified molecular markers.

Table IF shows the molecular markers that are differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member of the subgroup has obesity as compared to a second trait subgroup wherein each member of the second trait subgroup did not have obesity using the ChondroChip™ platform. The table also shows the sequences of selected products of the identified molecular markers.

Table IG shows the molecular markers that are differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member of the subgroup has hypertension and OA when compared with a second trait subgroup wherein each member of the second trait subgroup have OA only wherein molecular markers identified in Table IA have been removed so as to identify molecular markers which are unique to hypertension. The table also shows the sequences of selected products of the identified molecular markers.

Table IH shows the molecular markers which were identified in Table IA which are shared with those molecular markers differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member of the subgroup has both hypertension and OA when compared with a second trait subgroup wherein each member of the second trait subgroup have OA only. The table also shows the sequences of selected products of the identified molecular markers.

Table II shows the molecular markers that are differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member of the subgroup has both obesity and have OA when compared with a second trait subgroup wherein each member of the second trait subgroup have OA only and wherein molecular markers identified in Table IB have been removed so as to identify molecular markers which are unique to obesity. The table also shows the sequences of selected products of the identified molecular markers.

Table IJ shows the molecular markers identified in Table IB which are shared with those molecular markers differentially expressed in blood samples from patients who are obese and have OA when compared with patients who have OA. The table also shows the sequences of selected products of the identified molecular markers.

Table IK shows the molecular markers that are differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member of the subgroup has both allergies and OA when compared with a second trait subgroup

wherein each member of the second trait subgroup have OA only wherein molecular markers identified in Table 1C have been removed so as to identify molecular markers which are unique to allergies. The table also shows the sequences of selected products of the identified molecular markers.

5 **Table 1L** shows the identify of those molecular markers identified in Table 3C which are shared with those molecular markers differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member of the subgroup has both allergies and OA when compared with a second trait subgroup wherein each member of the second trait subgroup having OA only. The table also shows the
10 sequences of selected products of the identified molecular markers.

Table 1M shows the molecular markers that are differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member of the subgroup is taking systemic steroids and has OA when compared with a second trait subgroup wherein each member of the second trait subgroup have OA only wherein
15 molecular markers identified in Table 1D have been removed so as to identify molecular markers which are unique to patients on systemic steroids. The table also shows the sequences of selected products of the identified molecular markers.

Table 1N shows the identify of those molecular markers identified in Table 1D which are shared with those molecular markers differentially expressed in blood samples
20 from a training population comprised of a first trait subgroup where each member of the subgroup who are on systemic steroids and have OA when compared with a second trait subgroup wherein each member of the second trait subgroup have OA only. The table also shows the sequences of selected products of the identified molecular markers.

Table 1O shows the molecular markers that are differentially expressed in blood
25 from a training population comprised of a first trait subgroup where each member of the subgroup are either taking birth control, on prednisone or on hormone replacement therapy and presenting with OA using the ChondroChip™ platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 1P shows the molecular markers that are differentially expressed in blood
30 samples from a training population comprised of a first trait subgroup where each member of the subgroup has both type II diabetes as compared to a second trait subgroup wherein each member of the second trait subgroup does not have type II diabetes using the ChondroChip™ platform. The table also shows the sequences of selected products of the identified molecular markers.

Table IQ shows the molecular markers that are differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member of the subgroup has Hyperlipidemia as compared to a second trait subgroup wherein each member of the second trait subgroup does not have Hyperlipidemia using the

5 ChondroChip™ platform. The table also shows the sequences of selected products of the identified molecular markers.

Table IR shows the molecular markers that are differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member of the subgroup has lung disease as compared to a second trait subgroup wherein each

10 member of the second trait subgroup does not have lung disease using the ChondroChip™ platform. The table also shows the sequences of selected products of the identified molecular markers.

Table IS shows the molecular markers that are differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member

15 of the subgroup has bladder cancer as compared to a second trait subgroup wherein each member of the second trait subgroup does not have bladder cancer using the ChondroChip™ platform. The table also shows the sequences of selected products of the identified molecular markers.

Table IT shows the molecular markers that are differentially expressed in blood

20 samples from a training population comprised of a first trait subgroup where each member of the subgroup has early stage bladder cancer, late stage bladder cancer with a second trait subgroup wherein each member of the second trait subgroup does not have bladder cancer using the ChondroChip™ platform. The table also shows the sequences of selected products of the identified molecular markers.

Table IU shows the molecular markers that are differentially expressed in blood

25 samples from a training population comprised of a first trait subgroup where each member of the subgroup has coronary artery disease (CAD) as compared to a second trait subgroup wherein each member of the second trait subgroup does not have not having CAD using the ChondroChip™ platform. The table also shows the sequences of selected products of

30 the identified molecular markers.

Table IV shows the molecular markers that are differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member of the subgroup has rheumatoid arthritis as compared to a second trait subgroup wherein each member of the second trait subgroup does not have rheumatoid arthritis using the

ChondroChip™ platform . The table also shows the sequences of selected products of the identified molecular markers.

5 **Table IW** shows the molecular markers that are differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member of the subgroup has rheumatoid arthritis as compared to a second trait subgroup wherein each member of the second trait subgroup does not have rheumatoid arthritis using the Affymetrix® platform . The table also shows the sequences of selected products of the identified molecular markers.

10 **Table IX** shows the molecular markers that are differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member of the subgroup has depression as compared with a second trait subgroup wherein each member of the second trait subgroup does not having depression using the ChondroChip™ platform. The table also shows the sequences of selected products of the identified molecular markers.

15 **Table IY** shows the molecular markers that are differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member of the subgroup has one of various stages of osteoarthritis as compared with a second trait subgroup wherein each member of the second trait subgroup does not have osteoarthritis using the ChondroChip™ platform. The table also shows the sequences of selected products of the identified molecular markers.

20 **Table IZ** shows the molecular markers that are differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member of the subgroup has liver cancer as compared with a second trait subgroup wherein each member of the second trait subgroup does not have liver cancer using the ChondroChip™ platform. The table also shows the sequences of selected products of the identified molecular markers.

25 **Table IZ(B)** shows the molecular markers that are differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member of the subgroup has liver cancer as compared with a second trait subgroup wherein each member of the second trait subgroup does not have liver cancer using the Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

30 **Table IAA** shows the molecular markers that are differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member

of the subgroup has schizophrenia as compared with a second trait subgroup wherein each member of the second trait subgroup does not have schizophrenia using the Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

5 **Table IAB** shows the molecular markers that are differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member of the subgroup has Chagas disease as compared with a second trait subgroup wherein each member of the second trait subgroup does not have Chagas disease using the Affymetrix® platform. The table also shows the sequences of selected products of the
10 identified molecular markers.

Table IAC shows the molecular markers that are differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member of the subgroup has both asthma and osteoarthritis as compared a second trait subgroup wherein each member of the second trait subgroup has only osteoarthritis using the
15 ChondroChip™. The table also shows the sequences of selected products of the identified molecular markers.

Table IAD shows the molecular markers that are differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member of the subgroup has asthma as compared with a second trait subgroup wherein each
20 member of the second trait subgroup does not have asthma using the Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

Table IAE shows the molecular markers that are differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member
25 of the subgroup has lung cancer as compared with a second trait subgroup wherein each member of the second trait subgroup does not have lung cancer using the Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

Table IAG shows the molecular markers that are differentially expressed in blood
30 samples from a training population comprised of a first trait subgroup where each member of the subgroup has hypertension as compared with a second trait subgroup wherein each member of the second trait subgroup does not have hypertension using the Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

Table IAH shows the molecular markers that are differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member of the subgroup has obesity as compared with a second trait subgroup wherein each member of the second trait subgroup does not have obesity using the Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

Table IAI shows the molecular markers that are differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member of the subgroup has ankylosing spondylitis as compared with a second trait subgroup wherein each member of the second trait subgroup does not have ankylosing spondylitis using the Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 2 shows the molecular markers that are differentially expressed in blood from a training population comprised of a first trait subgroup where each member of the subgroup has either mild or severe OA, but for which molecular markers relevant to asthma, obesity, hypertension, systemic steroids and allergies have been removed. The table also shows the sequences of selected products of the identified molecular markers.

Table 3 is a group of tables wherein each table shows those molecular markers that are differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member of the subgroup has a first disease as compared to blood samples from a second trait subgroup wherein each member of the second trait subgroup has a second disease so as to allow differential diagnosis as between said first and second disease.

Table 3A shows the molecular markers that are differentially expressed in blood from a training population comprised of a first trait subgroup where each member of the subgroup has schizophrenia as compared with a second trait subgroup wherein each member of the second trait subgroup has manic depression syndrome (MDS) using the Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 3B shows the molecular markers that are differentially expressed in blood from a training population comprised of a first trait subgroup where each member of the subgroup has hepatitis as compared with a second trait subgroup wherein each member of the second trait subgroup has liver cancer using the Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 3C shows the molecular markers that are differentially expressed in blood from a training population comprised of a first trait subgroup where each member of the subgroup has bladder cancer as compared with a second trait subgroup wherein each member of the second trait subgroup has liver cancer using the Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 3D shows the molecular markers that are differentially expressed in blood from a training population comprised of a first trait subgroup where each member of the subgroup has bladder cancer as compared with a second trait subgroup wherein each member of the second trait subgroup has testicular cancer using the Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 3E shows the molecular markers that are differentially expressed in blood from a training population comprised of a first trait subgroup where each member of the subgroup has testicular cancer as compared with a second trait subgroup wherein each member of the second trait subgroup has kidney cancer using the Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 3F shows the molecular markers that are differentially expressed in blood from a training population comprised of a first trait subgroup where each member of the subgroup has liver cancer as compared with a second trait subgroup wherein each member of the second trait subgroup has stomach cancer using the Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 3G shows the molecular markers that are differentially expressed in blood from a training population comprised of a first trait subgroup where each member of the subgroup has liver cancer as compared with a second trait subgroup wherein each member of the second trait subgroup has colon cancer using the Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 3H shows the molecular markers that are differentially expressed in blood from a training population comprised of a first trait subgroup where each member of the subgroup has stomach cancer as compared with a second trait subgroup wherein each member of the second trait subgroup has colon cancer using the Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 3I shows the molecular markers that are differentially expressed in blood from a training population comprised of a first trait subgroup where each member of the subgroup has Rheumatoid Arthritis as compared with a second trait subgroup wherein each member of the second trait subgroup has Osteoarthritis using the Affymetrix® platform.

5 The table also shows the sequences of selected products of the identified molecular markers.

Table 3K shows the molecular markers that are differentially expressed in blood from a training population comprised of a first trait subgroup where each member of the subgroup has Chagas Disease as compared with a second trait subgroup wherein each member of the second trait subgroup has Heart Failure using the Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 3L shows the molecular markers that are differentially expressed in blood from a training population comprised of a first trait subgroup where each member of the subgroup has Chagas Disease as compared with a second trait subgroup wherein each member of the second trait subgroup has Coronary Artery Disease using the Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 3N shows the molecular markers that are differentially expressed in blood from a training population comprised of a first trait subgroup where each member of the subgroup has Coronary Artery Disease as compared with a second trait subgroup wherein each member of the second trait subgroup has Heart Failure using the Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 3P shows the molecular markers that are differentially expressed in blood from a training population comprised of a first trait subgroup where each member of the subgroup has Asymptomatic Chagas Disease as compared with a second trait subgroup wherein each member of the second trait subgroup has Symptomatic Chagas Disease using the Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 3Q shows the molecular markers that are differentially expressed in blood from a training population comprised of a first trait subgroup where each member of the subgroup has Alzheimer's' as compared with a second trait subgroup wherein each member of the second trait subgroup has Schizophrenia using the Affymetrix® platform.

The table also shows the sequences of selected products of the identified molecular markers.

Table 3R shows the molecular markers that are differentially expressed in blood from a training population comprised of a first trait subgroup where each member of the subgroup has Alzheimer's' as compared with a second trait subgroup wherein each member of the second trait subgroup has Manic Depression Syndrome using the Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 4 tables are those which shows molecular markers that are differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member of the subgroup has a stage of Osteoarthritis as compared to blood samples from a second trait subgroup wherein each member of the second trait subgroup has a second stage of Osteoarthritis so as to allow monitoring of progression and/or regression of disease. Each table also shows the sequences of selected products of the identified molecular markers.

Table 4A shows the molecular markers that are differentially expressed in blood from a training population comprised of a first trait subgroup where each member of the subgroup has Osteoarthritis as compared with a second trait subgroup wherein each member of the second trait subgroup is without Osteoarthritis using the ChondroChip™ platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 4B shows the molecular markers that are differentially expressed in blood from a training population comprised of a first trait subgroup where each member of the subgroup has Osteoarthritis as compared with a second trait subgroup wherein each member of the second trait subgroup is without Osteoarthritis using the Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 4C shows the molecular markers that are differentially expressed in blood from a training population comprised of a first trait subgroup where each member of the subgroup has mild Osteoarthritis as compared with a second trait subgroup wherein each member of the second trait subgroup is without mild Osteoarthritis using the ChondroChip™ platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 4D shows the molecular markers that are differentially expressed in blood from a training population comprised of a first trait subgroup where each member of the subgroup has mild Osteoarthritis as compared with a second trait subgroup wherein each member of the second trait subgroup is without Osteoarthritis using the Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 4E shows the molecular markers that are differentially expressed in blood from a training population comprised of a first trait subgroup where each member of the subgroup has moderate Osteoarthritis as compared with patients without Osteoarthritis using the ChondroChip™ platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 4F shows the molecular markers that are differentially expressed in blood from a training population comprised of a first trait subgroup where each member of the subgroup has moderate Osteoarthritis as compared a second trait subgroup wherein each member of the second trait subgroup is without Osteoarthritis using the Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 4G shows the molecular markers that are differentially expressed in blood from a training population comprised of a first trait subgroup where each member of the subgroup has marked Osteoarthritis as compared with a second trait subgroup wherein each member of the second trait subgroup is without Osteoarthritis using the ChondroChip™ platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 4H shows the molecular markers that are differentially expressed in blood from a training population comprised of a first trait subgroup where each member of the subgroup has marked Osteoarthritis as compared with a second trait subgroup wherein each member of the second trait subgroup is without Osteoarthritis using the Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 4I shows the molecular markers that are differentially expressed in blood from a training population comprised of a first trait subgroup where each member of the subgroup has severe Osteoarthritis as compared with a second trait subgroup wherein each member of the second trait subgroup is without Osteoarthritis using the ChondroChip™

platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 4J shows the molecular markers that are differentially expressed in blood from a training population comprised of a first trait subgroup where each member of the subgroup has severe Osteoarthritis as compared with a second trait subgroup wherein each member of the second trait subgroup is without Osteoarthritis using the Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 4K shows the molecular markers that are differentially expressed in blood from a training population comprised of a first trait subgroup where each member of the subgroup has mild Osteoarthritis as compared with a second trait subgroup wherein each member of the second trait subgroup has moderate Osteoarthritis using the ChondroChip™ platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 4L shows the molecular markers that are differentially expressed in blood from a training population comprised of a first trait subgroup where each member of the subgroup has mild Osteoarthritis as compared with a second trait subgroup wherein each member of the second trait subgroup has moderate Osteoarthritis using the Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 4M shows the molecular markers that are differentially expressed in blood from a training population comprised of a first trait subgroup where each member of the subgroup has mild Osteoarthritis as compared with a second trait subgroup wherein each member of the second trait subgroup has marked Osteoarthritis using the ChondroChip™ platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 4N shows the molecular markers that are differentially expressed in blood from a training population comprised of a first trait subgroup where each member of the subgroup has mild Osteoarthritis as compared with a second trait subgroup wherein each member of the second trait subgroup has marked Osteoarthritis using the Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 4O shows the molecular markers that are differentially expressed in blood from a training population comprised of a first trait subgroup where each member of the

subgroup has mild Osteoarthritis as compared with a second trait subgroup wherein each member of the second trait subgroup has severe Osteoarthritis using the ChondroChip™ platform. The table also shows the sequences of selected products of the identified molecular markers.

5 **Table 4P** shows the molecular markers that are differentially expressed in blood from a training population comprised of a first trait subgroup where each member of the subgroup has mild Osteoarthritis as compared with a second trait subgroup wherein each member of the second trait subgroup has severe Osteoarthritis using the Affymetrix® platform. The table also shows the sequences of selected products of the identified
10 molecular markers.

Table 4Q shows the molecular markers that are differentially expressed in blood from a training population comprised of a first trait subgroup where each member of the subgroup has moderate Osteoarthritis as compared with a second trait subgroup wherein each member of the second trait subgroup has marked Osteoarthritis using the
15 ChondroChip™ platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 4R shows the molecular markers that are differentially expressed in blood from a training population comprised of a first trait subgroup where each member of the subgroup has moderate Osteoarthritis as compared with a second trait subgroup wherein
20 each member of the second trait subgroup has marked Osteoarthritis using the Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 4S shows the molecular markers that are differentially expressed in blood from a training population comprised of a first trait subgroup where each member of the subgroup has moderate Osteoarthritis as compared with patients a second trait subgroup wherein each member of the second trait subgroup has severe Osteoarthritis using the
25 ChondroChip™ platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 4T shows the molecular markers that are differentially expressed in blood
30 from a training population comprised of a first trait subgroup where each member of the subgroup has moderate Osteoarthritis as compared with a second trait subgroup wherein each member of the second trait subgroup has severe Osteoarthritis using the Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 4U shows the molecular markers that are differentially expressed in blood from a training population comprised of a first trait subgroup where each member of the subgroup has marked Osteoarthritis as compared with a second trait subgroup wherein each member of the second trait subgroup has severe Osteoarthritis using the

5 ChondroChip™ platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 4V shows the molecular markers that are differentially expressed in blood from a training population comprised of a first trait subgroup where each member of the subgroup has marked Osteoarthritis as compared with a second trait subgroup wherein
10 each member of the second trait subgroup has severe Osteoarthritis using the Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 5 tables are those which identify molecular markers that are differentially expressed in blood samples from a training population comprised of a first trait subgroup
15 where each member of the subgroup has a disease or condition of interest as compared to blood samples from a second trait subgroup wherein each member of the second trait subgroup is without said disease or condition. The tables also shows the sequences of selected products of the identified molecular markers.

Table 5A shows the molecular markers that are differentially expressed in blood
20 samples from a training population comprised of a first trait subgroup where each member of the subgroup has psoriasis as compared with a second trait subgroup wherein each member of the second trait subgroup does not have psoriasis using the Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 5B shows the molecular markers that are differentially expressed in blood
25 samples from a training population comprised of a first trait subgroup where each member of the subgroup has thyroid disorder as compared with a second trait subgroup wherein each member of the second trait subgroup does not have thyroid disorder using the Affymetrix® platform. The table also shows the sequences of selected products of the
30 identified molecular markers.

Table 5C shows the molecular markers that are differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member of the subgroup has irritable bowel syndrome as compared with a second trait subgroup wherein each member of the second trait subgroup does not have irritable bowel syndrome

using the Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 5D shows the molecular markers that are differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member of the subgroup has osteoporosis as compared with a second trait subgroup wherein each member of the second trait subgroup does not have osteoporosis using the Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 5E shows the molecular markers that are differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member of the subgroup has migraine headaches as compared with a second trait subgroup wherein each member of the second trait subgroup does not have migraine headaches using the Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 5F shows the molecular markers that are differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member of the subgroup has eczema as compared with a second trait subgroup wherein each member of the second trait subgroup does not have eczema using the Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 5G shows the molecular markers that are differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member of the subgroup has NASH as compared with a second trait subgroup wherein each member of the second trait subgroup does not have NASH using the Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 5H shows the molecular markers that are differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member of the subgroup has Alzheimers' disease as compared with a second trait subgroup wherein each member of the second trait subgroup does not have Alzheimer's disease using the Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 5I shows the molecular markers that are differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member

of the subgroup has Manic Depression Syndrome as compared with a second trait subgroup wherein each member of the second trait subgroup does not have Manic Depression Syndrome using the Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

5 **Table 5J** shows the molecular markers that are differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member of the subgroup has Crohn's Colitis as compared with a second trait subgroup wherein each member of the second trait subgroup does not have Crohn's Colitis using the Affymetrix® platform. The table also shows the sequences of selected products of the
10 identified molecular markers.

Table 5K shows the molecular markers that are differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member of the subgroup has Chronic Cholecystitis as compared with a second trait subgroup wherein each member of the second trait subgroup does not have Chronic Cholecystitis
15 using the Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 5L shows the molecular markers that are differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member of the subgroup has Heart Failure as compared with a second trait subgroup wherein each
20 member of the second trait subgroup does not have Heart Failure using the Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 5M shows the molecular markers that are differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member
25 of the subgroup has Cervical Cancer as compared with a second trait subgroup wherein each member of the second trait subgroup does not have Cervical Cancer using the Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 5N shows the molecular markers that are differentially expressed in blood
30 samples from a training population comprised of a first trait subgroup where each member of the subgroup has Stomach Cancer as compared with a second trait subgroup wherein each member of the second trait subgroup does not have Stomach Cancer using the Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 5O shows the molecular markers that are differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member of the subgroup has Kidney Cancer as compared with a second trait subgroup wherein each member of the second trait subgroup does not have Kidney Cancer using the Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 5P shows the molecular markers that are differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member of the subgroup has Testicular Cancer as compared with a second trait subgroup wherein each member of the second trait subgroup does not have Testicular Cancer using the Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 5Q shows the molecular markers that are differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member of the subgroup has Colon Cancer as compared a second trait subgroup wherein each member of the second trait subgroup does not have Colon Cancer using the Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 5R shows the molecular markers that are differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member of the subgroup has Hepatitis B as compared with a second trait subgroup wherein each member of the second trait subgroup does not have Hepatitis B using the Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 5S shows the molecular markers that are differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member of the subgroup has Pancreatic Cancer as compared with a second trait subgroup wherein each member of the second trait subgroup does not have Pancreatic Cancer using the Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 5T shows the molecular markers that are differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member of the subgroup has Asymptomatic Chagas as compared with a second trait subgroup wherein each member of the second trait subgroup does not have Chagas using the

Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 5U shows the molecular markers that are differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member
5 of the subgroup has Symptomatic Chagas as compared with a second trait subgroup wherein each member of the second trait subgroup does not have Chagas using the Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 5V shows the molecular markers that are differentially expressed in blood
10 samples from a training population comprised of a first trait subgroup where each member of the subgroup has Bladder Cancer as compared with patients not having Bladder Cancer using the Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 6 tables are those tables which show those molecular markers that are
15 differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member of the subgroup has any one of a series of related conditions as compared to blood samples a second trait subgroup wherein each member of the second trait subgroup does not have said related conditions. The table also shows the sequences of selected products of the identified molecular markers.

Table 6A shows the molecular markers that are differentially expressed in blood
20 samples from a training population comprised of a first trait subgroup where each member of the subgroup has Cancer as compared with a second trait subgroup wherein each member of the second trait subgroup does not have Cancer using the Affymetrix® platform. The table also shows the sequences of selected products of the identified
25 molecular markers.

Table 6B shows the molecular markers that are differentially expressed in blood
samples from a training population comprised of a first trait subgroup where each member of the subgroup with Cardiovascular Disease as compared with a second trait subgroup wherein each member of the second trait subgroup does not have a Cardiovascular Disease
30 using the Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 6C shows the molecular markers that are differentially expressed in blood
samples from a training population comprised of a first trait subgroup where each member of the subgroup has a Neurological Disease as compared with a second trait subgroup

wherein each member of the second trait subgroup does not have a Neurological Disease using the Affymetrix® platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 7 tables are those tables which show molecular markers that are
5 differentially expressed in blood samples from with a condition wherein said condition is a treatment as compared to blood samples from patients without said treatment or with a different said treatment.

Table 7A shows the molecular markers that are differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member
10 of the subgroup is taking Celebrex® as compared a second trait subgroup wherein each member of the second trait subgroup is taking a Cox Inhibitor which was not Celebrex® using the ChondroChip™ platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 7B shows the molecular markers that are differentially expressed in blood
15 samples from a training population comprised of a first trait subgroup where each member of the subgroup is taking Celebrex® as compared with a second trait subgroup wherein each member of the second trait subgroup is not taking Celebrex® using the ChondroChip™ platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 7C shows the molecular markers that are differentially expressed in blood
20 samples from a training population comprised of a first trait subgroup where each member of the subgroup is taking Vioxx® as compared a second trait subgroup wherein each member of the second trait subgroup is not taking Vioxx® using the ChondroChip™ platform. The table also shows the sequences of selected products of the identified
25 molecular markers.

Table 7D shows the molecular markers that are differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member of the subgroup is taking Vioxx® as compared with a second trait subgroup wherein each member of the second trait subgroup on a Cox inhibitor but not on Vioxx® using the
30 ChondroChip™ platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 7E shows the molecular markers that are differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member of the subgroup is taking NSAIDS as compared with patients not on NSAIDS using the

ChondroChip™ platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 7F shows the molecular markers that are differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member of the subgroup is taking Cortisone as compared with a second trait subgroup wherein each member of the second trait subgroup on not on Cortisone using the ChondroChip™ platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 7G shows the molecular markers that are differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member of the subgroup is taking Visco Supplement as compared with a second trait subgroup wherein each member of the second trait subgroup not on Visco Supplement using the ChondroChip™ platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 7H shows the molecular markers that are differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member of the subgroup is taking Lipitor® as compared with a second trait subgroup wherein each member of the second trait subgroup not on Lipitor® using the ChondroChip™ platform. The table also shows the sequences of selected products of the identified molecular markers.

Table 7I shows the molecular markers that are differentially expressed in blood samples from a training population comprised of a first trait subgroup where each member of the subgroup is who are smokers as compared with a second trait subgroup wherein each member of the second trait subgroup who are not smokers using the ChondroChip™ platform. The table also shows the sequences of selected products of the identified molecular markers.

To further clarify, Tables IAA; IAB; IAD; IAE; IAG; IAH; IAT; IS; IT; IU; IW; IZ(b); 3A; 3B; 3C; 3D; 3E; 3F; 3G; 3H; 3I; 3K; 3L; 3P; 3Q; 3R; 4B; 4D; 4F; 4H; 4J; 4L; 4N; 4P; 4R; 4T; 4V; 5A; 5B; 5C; 5D; 5EE; 5F; 5G; 5H; 5I; 5J; 5K; 5L; 5M; 5N; 5O; 5P 5Q; 5R; 5S; 5T; 5U; 5V; 6A; 6B; 6C; 7F; and 7G each identify the molecular markers identified using the Affymetrix® genechip to screen the products of the majority of molecular markers of the human genome in accordance with step 202.

Tables IA; 1 AC; IB; 1C; ID; IE; IF; IG; IH; II; U; IK; IL; IM; IN; 10; IP; IQ; IR; IV; IX; IY; IZ; 2; 4A; 4C; 4E; 4G; 4I; 4K; 4M; 4O; 4Q; 4S; 4V; 7A; 7B; 7C;

7D; 7E; 7H; and 7I each identify molecular markers identified using our own ChondroChip™ genechip to screen the products of the majority of the molecular markers of the human genome.

Like reference numerals refer to corresponding parts throughout the several views
5 of the drawings.

5. DETAILED DESCRIPTION

The embodiments of the present invention use novel approaches to screen and select molecular markers and develop classifiers that can be used to harness the use of molecular marker data from blood. The present invention thus provides systems and
10 methods for constructing biological classifiers using molecular marker data from blood by providing a method to screen and select from a large variety of potential molecular markers so as to identify a small subset of molecular markers. The classifiers and the combinations of molecular markers identified using aspects of the current invention are useful for a wide variety of purposes including: diagnosis; prognosis; prediction of disease,
15 stage of disease or disease risk; monitoring disease progression and/or regression; monitoring disease reoccurrence and identifying risk of disease reoccurrence; determining and/or predicting response to treatment and/or treatment outcomes; monitoring and/or predicting treatment compliance or non-compliance and the like. As used herein, a "condition" includes a mode or state of being including a physical, emotional,
20 psychological or pathological state. A condition can be as a result of both "genetic" (ie genetically inherited) and/or "environmental" factors (ie the result of exposure to internal or external influences). In one embodiment of the invention, a condition is a disease. In another embodiment of the invention, a condition is a stage of a disease. In yet another embodiment of the invention, a condition is a mode or state of being which is not a
25 disease. For example in one embodiment, a condition which is not a disease is a condition resulting from the progression of time. A condition resulting from progression of time can include, but is not limited to: memory loss, loss of skin elasticity, loss of muscle tone, and loss of sexual desire. In a further embodiment of the invention a condition which is not a disease is the response to treatment. A treatment can include, but is not limited to disease
30 modifying treatments as well as treatments useful in mitigating the symptoms of disease. For example treatments can include drugs specific for a disease of the invention.

As used herein, the term "data" or "molecular marker data" generally refers to data reflective of the abundance of a product of a molecular marker in blood including either or both of RNA and protein.

As used herein, "diagnosis" includes the ability to determine that an individual has or does not have a specific condition or conditions. Diagnosis also refers to the ability to determine that an individual has one condition or conditions as compared with one or more other condition or conditions. In some embodiments, diagnosis refers to the ability to demonstrate an increased likelihood that an individual has a specific condition. "diagnosis" refers to the ability to demonstrate an increased likelihood that an individual has one condition as compared to a second condition. More particularly "diagnosis" refers to a process whereby there is an increased likelihood that an individual is properly characterized as having a condition ("true positive") or is properly characterized as not having a condition (or is properly characterized as having the second condition where the diagnosis is as between two conditions) ("true negative") while minimizing the likelihood that the individual is improperly characterized with said condition ("false positive") or improperly characterized as not being afflicted with said condition (or improperly characterized as having the second condition)("false negative").

As used herein, the term "differential expression" refers to a difference in the level of expression of the RNA and/or protein products of a molecular marker of the invention, as measured by the amount or level of RNA or protein. In reference to RNA, it can include difference in the level of expression of mRNA, and/or one or more spliced variants of mRNA of the biomarker in one sample as compared with the level of expression of the same one or more biomarkers of the invention as measured by the amount or level of RNA, including mRNA and/or one or more spliced variants of mRNA in a second sample. "Differentially expressed" or "differential expression" can also include a measurement of the protein, or one or more protein variants encoded by a molecular marker of the invention in a sample or population of samples as compared with the amount or level of protein expression, including one or more protein variants of a molecular marker of the invention. Differential expression can be determined as described herein and as would be understood by a person skilled in the art. The term "differentially expressed" or "changes in the level of expression" refers to an increase or decrease in the measurable expression level of a given product of a molecular marker as measured by the amount of RNA and/or the amount of protein in a sample as compared with the measurable expression level of a given product of the molecular marker in a second sample. The first sample and second sample need not be from different patients, but can be samples from the same patient taken at different time points. The term "differentially expressed" or "changes in the level of expression" can also refer to an increase or decrease in the measurable expression level of

a given molecular marker in a population of samples as compared with the measurable expression level of the molecular marker in a second population of samples. As used herein, "differentially expressed" when referring to a single sample can be measured using the ratio of the level of expression of a given molecular marker in said sample as compared

5 with the mean expression level of the given molecular marker of a control population wherein the ratio is not equal to 1.0. Differentially expressed can also be used to include comparing a first population of samples as compared with a second population of samples or a single sample to a population of samples using either a ratio of the level of expression or using p-value. When using p-value, a nucleic acid transcript including hnRNA and

10 mRNA is identified as being differentially expressed as between a first and second population when the p-value is less than 0.1, less than 0.05, less than 0.01, less than 0.005, less than 0.001 etc. When determining differential expression on the basis of the ratio of the level of molecular marker product—expression of an RNA or protein product of the molecular marker is differentially expressed if the ratio of the level of the RNA or protein

15 product in a first sample as compared with that in a second sample is greater than or less than 1.0. For instance, a ratio of greater than 1, for example 1.2, 1.5, 1.7, 2, 3, 4, 10, 20, or a ratio of less than 1, for example 0.8, 0.6, 0.4, 0.2, 0.1. 0.05, of RNA or protein product of a molecular marker would be indicative of differential expression. In another embodiment of the invention, a molecular marker is differentially expressed if the mean

20 level of expression of a nucleic acid transcript including the hnRNA and/or mRNA transcript in a first population as compared with its mean level of expression of the transcript in a second population is greater than or less than 1.0. For instance, a ratio of greater than 1, for example 1.2, 1.5, 1.7, 2, 3, 4, 10, 20, or a ratio less than 1, for example 0.8, 0.6, 0.4, 0.2, 0.1. 0.05 would be indicative of differential expression. In another

25 embodiment of the invention a molecular marker is differentially expressed if the ratio of the level of the hnRNA and/or mRNA transcript in a first sample as compared with the mean level of the transcript of the second population is greater than or less than 1.0 and includes for example, a ratio of greater than 1, for instance 1.2, 1.5, 1.7, 2, 3, 4, 10, 20, or a ratio less than 1, for example 0.8, 0.6, 0.4, 0.2, 0.1. 0.05. "Differentially increased

30 expression" refers to 1.1 fold, 1.2 fold, 1.4 fold, 1.6 fold, 1.8 fold, or more, relative to a standard, such as the mean of the expression level of the second population. "Differentially decreased expression" refers to less than 1.0 fold, 0.8 fold, 0.6 fold, 0.4 fold, 0.2 fold, 0.1 fold or less, relative to a standard, such as the mean of the expression level of the second population.

As used herein, the term "molecular marker" (or sometimes referred to as a "biomarker") refers to a gene or a genetic element. In some embodiments, the molecular marker of interest is identified by the Gene ID (formerly Locus Link ID) as is published by the National Center for Biotechnology Information (NCBI) Database as would be
5 understood by a person skilled in the art.

As used herein, the term "oligonucleotide" is defined as a molecule comprised of two or more deoxyribonucleotides and/ or ribonucleotides, and preferably more than three. Its exact size will depend upon many factors which, in turn, depend upon the ultimate function and use of the oligonucleotide. The oligonucleotides may be from about 8 to
10 about 1,000 nucleotides long. Although oligonucleotides of 8 to 100 nucleotides are useful in the invention, preferred oligonucleotides range from about 8 to about 15 bases in length, from about 8 to about 20 bases in length, from about 8 to about 25 bases in length, from about 8 to about 30 bases in length, from about 8 to about 40 bases in length or from about 8 to about 50 bases in length.

15 The term, "primer", as used herein refers to an oligonucleotide, whether occurring naturally as in a purified restriction digest or produced synthetically, which is capable of acting as a point of initiation of synthesis when placed under conditions in which synthesis of a primer extension product, which is complementary to a nucleic acid strand, is induced, i.e., in the presence of nucleotides and an inducing agent such as a DNA polymerase and at
20 a suitable temperature and pH. The primer may be either single-stranded or double-stranded and must be sufficiently long to prime the synthesis of the desired extension product in the presence of the inducing agent. The exact length of the primer will depend upon many factors, including temperature, source of primer and the method used. For example, for diagnostic applications, depending on the complexity of the target sequence,
25 the oligonucleotide primer typically contains 15-25 or more nucleotides, although it may contain fewer nucleotides. The factors involved in determining the appropriate length of primer are readily known to one of ordinary skill in the art. In general, the design and selection of primers embodied by the instant invention is according to methods that are standard and well known in the art, see Dieffenbach, C.W., Lowe, T.M.J., Dveksler, G.S.
30 (1995) General Concepts for PCR Primer Design. In: PCR Primer, A Laboratory Manual (Eds. Dieffenbach, CW, and Dveksler, G.S.) Cold Spring Harbor Laboratory Press, New York, 133-155; Innis, M.A., and Gelfand, D.H. (1990) Optimization of PCRs. In: PCR protocols, A Guide to Methods and Applications (Eds. Innis, M.A., Gelfand, D.H., Sninsky, J.J., and White, T.J.) Academic Press, San Diego, 3-12; Sharrocks, A.D. (1994)

The design of primers for PCR. In: PCR Technology, Current Innovations (Eds. Griffin, H.G., and Griffin, A.M, Ed.) CRC Press, London, 5-11.

As used herein, the term "probe" means oligonucleotides and analogs thereof and refers to a range of chemical species that recognise polynucleotide target sequences through hydrogen bonding interactions with the nucleotide bases of the target sequences. The probe or the target sequences may be single- or double-stranded RNA or single- or double-stranded DNA or a combination of DNA and RNA bases. A probe is at least 8 nucleotides in length and less than the length of a complete gene. A probe may be 10, 20, 30, 50, 75, 100, 150, 200, 250, 400, 500 and up to 2000 nucleotides in length. Probes can include oligonucleotides modified so as to have a tag which is detectable by fluorescence, chemiluminescence and the like. The probe can also be modified so as to have both a detectable tag and a quencher molecule, for example Taqman® and Molecular Beacon® probes.

As used herein, the term "product of the molecular marker" or "molecular marker product" refers to the RNA or protein found in blood which corresponds to the molecular marker (ie is transcribed from the gene or genetic element or is translated from RNA which is transcribed from the gene or genetic element). For example, in some embodiments RNA resulting from the molecular marker can include one or more of the following species; hnRNA, niRNA, and/or one or more spliced variants of mRNA. In some embodiments, proteins resulting from the molecular marker can include any proteins found in blood which correspond to the RNA resulting from the molecular marker.

As used herein, the term "selectively amplified" or "selective amplification", refers to a process whereby one or more copies of a particular target nucleic acid sequence is selectively generated from a template nucleic acid. Selective amplification or selectively amplified is to be compared with amplification in general which can be used as a method in combination with, for example, random primers and an oligodT primer to amplify a population of nucleic acid sequences (e.g. mRNA). Selective amplification is preferably done by the method of polymerase chain reaction (Mullis and Faloona, 1987, Methods Enzymol. 155:335).

As used herein, the term "selectively binds" in the context of proteins encompassed by the invention refers to the specific interaction of any two of a peptide, a protein, a polypeptide, and an antibody, wherein the interaction preferentially occurs as between any two of a peptide, protein, polypeptide and antibody preferentially as compared with any other peptide, protein, polypeptide and antibody. For example, when the two molecules

are protein molecules, a structure on the first molecule recognises and binds to a structure on the second molecule, rather than to other proteins. "Selective binding", "Selective binding", as the term is used herein, means that a molecule binds its specific binding partner with at least 2-fold greater affinity, and preferably at least 10-fold, 20-fold, 50-fold, 100-fold or higher affinity than it binds a non-specific molecule.

As used herein "selective hybridization" in the context of this invention refers to a hybridization which occurs as between a polynucleotide encompassed by the invention and an RNA, and its complement thereof (ie a cDNA copy), of the molecular marker of the invention, wherein the hybridization is such that the polynucleotide preferentially binds to the RNA products of the molecular marker of the invention relative to the RNA products of other molecular markers or other genes in the genome in question. In a preferred embodiment a polynucleotide which "selectively hybridizes" is one which hybridizes with a selectivity of greater than 70%, greater than 80%, greater than 90% and most preferably of 100% (i.e. cross hybridization with other RNA species preferably occurs at less than 30%, less than 20%, less than 10%). As would be understood to a person skilled in the art, a polynucleotide which "selectively hybridizes" to the RNA product of a biomarker of the invention can be determined taking into account the length and composition.

As used herein, "specifically hybridizes", "specific hybridization" refers to hybridization which occurs when two nucleic acid sequences are substantially complementary (at least about 65% complementary over a stretch of at least 14 to 25 nucleotides, preferably at least about 75% complementary, more preferably at least about 90% complementary). See Kanehisa, M., 1984, Nucleic acids Res., 12:203, incorporated herein by reference. As a result, it is expected that a certain degree of mismatch is tolerated. Such mismatch may be small, such as a mono-, di- or tri-nucleotide. Alternatively, a region of mismatch can encompass loops, which are defined as regions in which there exists a mismatch in an uninterrupted series of four or more nucleotides. Numerous factors influence the efficiency and selectivity of hybridization of two nucleic acids, for example, the hybridization of a nucleic acid member on an array to a target nucleic acid sequence. These factors include nucleic acid member length, nucleotide sequence and/or composition, hybridization temperature, buffer composition and potential for steric hindrance in the region to which the nucleic acid member is required to hybridize. A positive correlation exists between the nucleic acid length and both the efficiency and accuracy with which a nucleic acid will anneal to a target sequence. In particular, longer sequences have a higher melting temperature (TM) than do shorter ones,

and are less likely to be repeated within a given target sequence, thereby minimizing non-specific hybridization. Hybridization temperature varies inversely with nucleic acid member annealing efficiency. Similarly the concentration of organic solvents, e.g., formamide, in a hybridization mixture varies inversely with annealing efficiency, while
5 increases in salt concentration in the hybridization mixture facilitate annealing. Under stringent annealing conditions, longer nucleic acids, hybridize more efficiently than do shorter ones, which are sufficient under more permissive conditions.

As used herein, the term "specifically binds" refers to the interaction of two molecules, e.g., a ligand and a protein or peptide, or an antibody and a protein or peptide
10 wherein the interaction is dependent upon the presence of particular structures on the respective molecules. For example, when the two molecules are protein molecules, a structure on the first molecule recognises and binds to a structure on the second molecule, rather than to proteins in general. "Specific binding", as the term is used herein, means that a molecule binds its specific binding partner with at least 2-fold greater affinity, and
15 preferably at least 10-fold, 20-fold, 50-fold, 100-fold or higher affinity than it binds a non-specific molecule.

As herein used, the term "standard stringent conditions" and "stringent conditions" means hybridization will occur only if there is at least 95% and preferably, at least 97% identity between the sequences, wherein the region of identity comprises at least 10
20 nucleotides. In one embodiment, the sequences hybridize under stringent conditions following incubation of the sequences overnight at 42°C, followed by stringent washes (0.2X SSC at 65°C). The degree of stringency of washing can be varied by changing the temperature, pH, ionic strength, divalent cation concentration, volume and duration of the washing. For example, the stringency of hybridization may be varied by conducting the
25 hybridization at varying temperatures below the melting temperatures of the probes. The melting temperature of the probe may be calculated using the following formulas:

For oligonucleotide probes, between 14 and 70 nucleotides in length, the melting temperature (T_m) in degrees Celcius may be calculated using the formula:

$T_m = 81.5 + 16.6(\log [Na^+]) + 0.41(\text{fraction G+C}) - (600/N)$ where N is the length of the

30 oligonucleotide.

For example, the hybridization temperature may be decreased in increments of 5°C from 68°C to 42°C in a hybridization buffer having a Na⁺ concentration of approximately 1M. Following hybridization, the filter may be washed with 2X SSC, 0.5% SDS at the

temperature of hybridization. These conditions are considered to be "moderate stringency" conditions above 50°C and "low stringency" conditions below 50°C. A specific example of "moderate stringency" hybridization conditions is when the above hybridization is conducted at 55°C. A specific example of "low stringency" hybridization conditions is
5 when the above hybridization is conducted at 45°C.

If the hybridization is carried out in a solution containing formamide, the melting temperature of the annealing nucleic acid strands may be calculated using the equation $T_m = 81.5 + 16.6(\log [Na^+]) + 0.41(\text{fraction G + C}) - (0.63\% \text{ formamide}) - (600/N)$, where N is the length of the probe.

10 If the hybridization is carried out in a solution containing formamide, the melting temperature of the annealing nucleic acid strands may be calculated using the equation $T_m = 81.5 + 16.6(\log [Na^+]) + 0.41(\text{fraction G + C}) - (0.63\% \text{ formamide}) - (600/N)$, where N is the length of the probe.

For example, the hybridization may be carried out in buffers, such as 6X SSC,
15 containing formamide at a temperature of 42°C. In this case, the concentration of formamide in the hybridization buffer may be reduced in 5% increments from 50% to 0% to identify clones having decreasing levels of homology to the probe. Following hybridization, the filter may be washed with 6X SSC, 0.5% SDS at 50 DC. Hybridization conditions are considered to be "moderate stringency" conditions when hybridization
20 fluids are comprised of above 25% formamide and "low stringency" conditions when hybridization fluids are comprised of below 25% formamide. A specific example of "moderate stringency" hybridization conditions is when the above hybridization is conducted at 30% formamide. A specific example of "low stringency" hybridization conditions is when the above hybridization is conducted at 10% formamide.

25 As used herein, the term "responder" is used to mean an individual who responds to treatment. The use of the term "responds to treatment" depends upon the context of the treatment and the disease or condition, but in some embodiments indicate a sufficiently effective and safe response by an individual to the administration of treatment.

As used herein, the term "non-responder" is used to mean an individual who does
30 not respond positively to treatment. The use of the phrase "does not respond to treatment" also depends upon the context of the treatment and the disease or condition, but in some embodiments indicates an ineffective or unsafe response by an individual to the administration of treatment.

As used herein, the term "trait" is a mode or state of being including a physical, emotional, psychological or pathological state. A trait can include both "genetic" and/or "environmentally" influenced factors. The term "genetic factors" means genetically inherited elements which affect one or more traits as a result of the genetic makeup of the individual. The term "environmental factors" includes exposure to internal or external influences including but not limited to medical treatments, non-medical drugs, pollution, environmental toxins, lead poisoning, mercury poisoning, exposure to genetically modified organisms, radioactivity, pesticides, insecticides, cigarette smoke, alcohol, or exercise and can affect abundance of RNA or affect gene expression as a result of epigenetic mutations and/or non genetic mutations. A physiological or pathological trait can include the status with regards to a condition including having a condition including a disease, having risk factors of a disease having a certain stage of disease or having a certain response to treatment or a risk of a certain response to treatment. In some cases a displayed trait can actually be the result of one or more underlying traits. A trait also includes clinically measurable parameters including those clinically measurable parameters which are indicators of state of health or disease. For example, a clinically measurable parameter includes blood pressure, lung capacity, electrolyte level, enzyme levels (e.g. Serum Glutamic Oxaloacetic Transaminase, alkaline phosphatase, Gamma-Glutamyltransferase or Gamma-Glutamyl Transpeptidase, Lactic dehydrogenase) hormone levels (e.g. thyroid stimulating hormone); protein levels (e.g. Prostate specific antigen PSA) and the like. Clinically measurable parameters can include disease specific clinical indicators, for example prostate specific antigen as an indicator of prostate cancer; insulin levels as an indicator of diabetes; thyroid stimulating hormone levels as an indicator of thyroid disorder and the like.

As used herein, the term "trait subgroup" is used to define a group of subjects where each subject has at least one trait or group of traits in common, for example, each subject has a disease, a specific stage of disease, same response to treatment, taking the same drug, etc.

As used herein, the terms "treatment", "treat", and "treating" includes administration of one or more compounds, combination of one or more compounds, application of a non-compound based therapeutic regimen, or any combination thereof where administration includes application of a single treatment, a regiment or course of treatment etc. to reduce or amelioration of the progression, severity and/or duration of a

disease or condition and/or the reduction or amelioration of the symptoms of a disease or condition resulting from the use of a treatment and/or treatment regime.

5.1 INVENTIVE SYSTEMS AND ALGORITHMS

Fig. 1 shows an exemplary system according to an embodiment of the invention
5 that supports the functionality described herein. The system is preferably a computer system 10 having:

- one or more central processors 22;
- a main non-volatile storage unit 14, for example a hard disk drive, for storing software and data, the storage unit 14 controlled by storage
10 controller 12;
- a system memory 36, preferably high speed random-access memory (RAM), for storing system control programs, data, and application programs, comprising programs and data loaded from non-volatile storage unit 14; system memory 36 may also include read-only memory (ROM);
- 15 • an optional user interface 32, comprising one or more input devices (e.g., keyboard 28) and a display 26 or other output device;
- an optional network interface card 20 for connecting to any wired or wireless communication network 34 (e.g., a wide area network such as the Internet);
- 20 • an internal bus 30 for interconnecting the aforementioned elements of the system; and
- a power source 24 to power the aforementioned elements.

Operation of computer 10 is controlled primarily by operating system 40, which is executed by central processing unit 22. Operating system 40 can be stored in system
25 memory 36. In addition to operating system 40, in a typical implementation, system memory 36 includes various components described below. Those of skill in the art will appreciate that such components can be wholly resident in RAM 36 or non-volatile storage unit 14. Furthermore, at any given time, such components can partially reside both in RAM 36 and non-volatile storage unit 14. Further still, some of the components shown in
30 Fig. 1 as being resident in RAM 36 can be resident in another computer (a remote computer) that is addressable by computer 10 over wide area network 34. It will be appreciated that such a remote computer may physically be resident in the same room as computer 10 or in another physical location. As illustrated in Fig. 1, in one exemplary

embodiment of the invention, RAM 36 comprises programs and data to interact with components in the computer system 10 for configuring:

- file system 42 for controlling access to the various files and data structures used by embodiments of the present invention;
- 5 • a training population 44 for use in construction of one or more classifiers ;
- a molecular marker data processing module I- 54 for processing molecular marker data representative of a genome or a portion thereof for members of training population 44;
- 10 • a molecular marker screening module A (56A) for identifying molecular markers whose molecular marker data individually discriminates between two or more trait subgroups of the training population using molecular marker data of module 54;
- a molecular marker screening module B (56B) for identifying molecular markers whose molecular marker data do not individually discriminate, but
15 demonstrate ability to differentiate between two or more trait subgroups of the training population when used in combination using molecular marker data of module 54;
- a candidate molecular marker data structure 58 for storing information about candidate molecular markers identified by molecular marker
20 candidate screening module 56A and optionally molecular marker candidate screening module 56B;
- a second molecular marker data processing module II 61 for processing additional molecular marker data for a selection of the candidate molecular markers identified in screening module 56A and, optionally, screening
25 module 56B for members of a training population;
- an outlier selection module 57 for evaluating molecular marker data identified in either module 56A and/or 56B or module 61 so as to remove one or more individuals from the training population as outliers;
- a combination module 61-5 which selects combinations of molecular
30 markers from candidate molecular markers identified in module 56A and optionally module 56B
- a molecular marker classifier construction module 62 for constructing candidate classifiers from combinations of molecular markers identified by molecular marker combination module 61-5;

- a molecular marker classifier evaluation module 64 for evaluating and selecting candidate classifiers constructed by molecular marker construction module 62;
- a classifier polling and reporting module 66 for receiving patient or subject molecular marker data and polling one or more classifiers selected by evaluation module 64 in order to determine whether a patient or subject has the disease or trait associated with each of the respective classifiers;
- a patient database 68 for storage of molecular marker data for diagnostic, prognostic or predictive use; and
- a classifier database 70 for storage of one or more classifiers selected by molecular marker classifier evaluation module 64.

As illustrated in Fig. 1, computer 10 comprises software program modules and data structures. The data structures either stored in computer 10 or accessible to computer 10 include a training population 44, candidate molecular marker data structures 58, patient database 68, and classifier database 70. Each of these data structures can comprise any form of data storage system including, but not limited to, a flat ASCII or binary file, an Excel spreadsheet, a relational database (*e.g.* SQL), or an on-line analytical processing (OLAP) database (MDX and/or variants thereof). In some specific embodiments, such data structures are each in the form of one or more databases that include hierarchical structure (*e.g.*, a star schema). In some embodiments, such data structures are each in the form of databases that do not have explicit hierarchy (*e.g.*, dimension tables that are not hierarchically arranged).

In some embodiments, each of the data structures stored or accessible to system 10 are single data structures. In other embodiments, such data structures in fact comprise a plurality of data structures (*e.g.*, databases, files, archives) that may or may not all be hosted by the same computer 10. For example, in some embodiments, training population 44 comprises a plurality of Excel spreadsheets that are stored either on computer 10 and/or on computers that are addressable by computer 10 across wide area network 34. In another example, patient database 68 comprises a database that is either stored on computer 10 or is distributed across one or more computers that are addressable by computer 10 across wide area network 34. Section 5.9 describes exemplary architectures for training population 44, candidate molecular marker data structure 58, patient database 68, and/or classifier database 70.

It will be appreciated that many of the modules and data structures illustrated in Fig. 1 can be located on one or more remote computers. For example, some embodiments of the present application are web service-type implementations. In such embodiments, classifier polling and reporting module 66 and other modules can be used by a physician to
5 treat a patient and can reside on a client computer that is in communication with computer 10 via network 34. In some embodiments, for example, classifier polling and reporting module 66 can be an interactive web page.

In some embodiments, training population 44, candidate molecular marker data structure 58, patient database 68 and/or classifier database 70 and modules (*e.g.* modules
10 54, 56A, 56B, 57, 61, 61-5, 62, 64, and 66) illustrated in Fig. 1 are on a single computer (computer 10) and in other embodiments one or more of such data structures and module are hosted by one or more remote computers (not shown). Any arrangement of the data structures and software modules illustrated in Fig. 1 on one or more computers is within the scope of the present invention so long as these data structures and software modules
15 are addressable with respect to each other across network 34 or by other electronic means. Thus, the present invention fully encompasses a broad array of computer systems.

Now that an overview of a system in accordance with one embodiment of the present invention has been described, various advantageous methods in accordance with embodiments of the present invention will now be disclosed in conjunction with Figs. 2
20 through 5. Figure 2 is a flowchart showing a method of selecting molecular markers and developing one or more classifiers or groups of classifiers according to an embodiment of the invention.

Step 202.

Referring to Fig. 2A, in step 202, molecular marker data reflective of the
25 abundance of each of a plurality of RNA and/or proteins found in the blood for members of training population 44 is obtained using one or more of the techniques as described in Section 5.3 and/or 5.4. In some embodiments, the data is reflective of the abundance of RNA products of the molecular marker. In some embodiments, the RNA products are those expressed in blood. In other embodiments, the RNA products are those which are
30 found in blood, but may not necessarily be expressed in blood (*e.g.* in instances where sufficient mRNA is transported into the blood to be detected. In some embodiments, the data is reflective of the abundance of protein products of the molecular markers. In some embodiments, the data is reflective of the level of proteins expressed in blood. In other embodiments, the data is reflective of proteins found in blood in sufficient quantity to be

detected. Measuring of molecular marker data (ie data reflective of the level of the product of the molecular marker) can be done using those techniques known to persons skilled in the art. Note that in some embodiments, data may be obtained using public sources or other sources of data rather than performing one or more of the techniques described. For
5 example, it is anticipated that databases of microarray data collected from blood may be available in future.

In some embodiments, the molecular marker data for each molecular marker is obtained using the same technique to allow greater comparability. In some instances *a priori* information is known about all or a portion of such genes and in some embodiments
10 *apriori* information about such genes is either not known or not considered in step 202. In some embodiments the molecular markers resulting from step are those molecular markers identified in Tables IA through to 71.

Measurement of molecular marker data of a plurality of the molecular markers in the blood of each member of training population 44 can be done using any known
15 technique and preferably is done using large scale techniques which allow for the ability to obtain data for a large number of molecular markers and/or for a large number of individuals quickly and efficiently and at a relatively low cost. For example, microarray techniques and RT-PCR and/or Quantitative RT-PCR can be useful large scale techniques. For example, a high throughput or large scale technique is a technique which allows one to
20 obtain data for large numbers of genes concurrently e.g. 1,000 genes, 5,000 genes, 10,000 genes, 15,000 genes 30,000 genes; or all of the genes of the genome of interest. It is expected that additional techniques are being developed and are also useful in embodiments of the invention to screen large numbers of genes quickly and efficiently.

Training population 44 includes a population of individuals made up of one or
25 more trait subgroups with each individual in such trait subgroups having one or more traits.

In some embodiments, each trait subgroup represented in training population 44 includes molecular marker data from at least 3-4 different subjects. More preferably, each trait subgroup represented in training population 44 includes molecular marker data for at
30 least ten different subjects. Still more preferably each trait subgroup represented in the training population 44 includes molecular marker data for at least 30, 40, 50, 100, 200, 500, 1000 or more subjects.

Each training population is selected to include two or more trait subgroups, each subgroup comprising trait subgroup members. Each of these two or more trait subgroups

differs with respect to a trait of interest and/or an aspect of a trait of interest. In one embodiment, the members of each of the trait subgroups have been diagnosed as having or not having the trait of interest by one or more known techniques. In another embodiment, the members of each trait subgroup are diagnosed for having or not having the trait of interest using a well accepted methodology for diagnosing of said trait.

For example, each member of a first trait subgroup of the training population has liver cancer, whereas each member of a second trait subgroup of the training population does not have liver cancer. In another embodiment, each member of a first trait subgroup of the training population has Alzheimer's, whereas each member of a second trait subgroup of the training population has manic depressive disorder, and each member of a third trait subgroup of the training population has schizophrenia, and each member of a fourth trait subgroup does not have any of the above conditions. In another example, the trait of interest is a disease such as prostate cancer, and the aspect of interest is the degree of advancement of the prostate cancer. Thus, each member of the first trait subgroup can be those subjects that have early stage prostate cancer, each member of a second trait subgroup can be those subjects that have later stage prostate cancer and each member of a third trait subgroup can be those subjects that do not have prostate cancer. In another example, the trait of interest is responsiveness of individuals having musculoskeletal disorders to a Cox 2 inhibitor. A first trait subgroup is comprised of individuals who are responsive to a treatment, a second trait subgroup is comprised of individuals who are responsive to treatment but demonstrate a toxic side-effect, and a third trait subgroup is comprised of individuals who are nonresponsive to treatment. In another embodiment, one trait subgroup can include those subjects that have not yet undergone treatment but who are later identified as being responders to treatment and a second trait subgroup can include those subjects that have not yet undergone treatment but who are later identified as non responders (*e.g.* demonstrates a toxic side-effect, demonstrates no improvement in condition, demonstrates a worsening of condition, *etc.*).

In some embodiments, members of each trait subgroup of the training population are preferably selected such that each trait subgroup of the training population has a similar distribution with respect to at least one, two, three, four, five, six, one or more, two or more, three or more, four or more, five or more, six or more, between one and 1000 other traits. For example, age, sex, body mass index (BMI), genetic variation information (*e.g.*, gene SNP mutations, restriction fragment length polymorphisms, microsatellite markers, restriction fragment length polymorphisms, and presence, absence or

characterization of short tandem repeats.), treatment regimens; co-morbidities; concentrations of metabolites, blood chemistry levels, and/or other indicators of health and/or wellness.

A treatment can include, but is not limited to, disease modifying treatments as well
5 as treatments useful in mitigating the symptoms of disease and includes administration of one or more compounds, combinations of one or more compounds, application of a non-compound based therapeutic regimen, or any combination thereof where administration includes application of a single treatment, a regimen or course of treatment and the like. For example, treatments can include drugs specific for a disease such as drugs specific for
10 Alzheimer's, cardiovascular disease, manic depression syndrome, schizophrenia, diabetes cancers including liver cancer, testicular cancer, bladder cancer, prostate cancer, kidney cancer, breast cancer, colon cancer, osteoarthritis, rheumatoid arthritis, osteoporosis, ankylosing spondylitis, or any other disease including those listed herein. . For example, treatments can include but are not limited to administration of VIOXX®, Celebrex®, non-steroidal anti-inflammatory drugs (NSAIDS), cortisone, visco supplement, Lipitor®,
15 Adriamycin®, Cytosan®, Herceptin®, Nolvadex®, Avastin®, Erbitux®, Fluorouracil®, Largactil®, Sparine®, Vesprin®, Stelazine®, Fentazine®, Prolixin®, Compazine®, Tindal®, Modecate®, Moditen®, Mellarin, Serentil, Norvane, ®, Fluanxol®, Clopixol®, Taractan®, Depixol®, Clopixol®, Haldol®, Haldol®, Decanoate, Orap®, Inapsine®,
20 Imap®, Semap®, Loxitane®, Daxol®, lithium, anticonvulsants (e.g., carbamazepine), antidepressants, and/or Moban®. More generally, a treatment can include any treatment or drug described in the *Compendium of Pharmaceuticals and Specialties*, Canadian Pharmaceutical Association; 26th edition, June, 1991; Krogh, *Compendium of Pharmaceuticals and Specialties*, Canadian Pharmaceutical Association; 27th edition,
25 April, 1992. In another embodiment, a treatment can include administration of any compound described in the United States Food and Drug Administration list of approved drug products (the "Orange Book") that is found at <http://www.mco.edu/research/fda.html>.

In some embodiments, molecular marker data is not obtainable from each member of a training population or each member of a trait subgroup (for example, using microarray
30 technology there may be an insufficient signal for one or more molecular markers for any particular member of the training population). Nevertheless, as would be understood by a person skilled in the art, candidate molecular markers can still be selected on the basis of the molecular marker data so long as data is obtainable for a sufficient number of molecular markers from a sufficient number of members of the training population. For

example, for each molecular marker, it is sufficient if data is available for at least 75%, 80%, 85%, 90% or 95% of the each trait subgroup of the training population.

Section 5.2 provides details on the types of blood samples from subjects in the training population that can be used to obtain data for molecular markers. Section 5.2
5 further provides details on how such blood samples can be obtained. Section 5.2 also provides details on the types of subjects that can be used to form training population 44 and the types of subpopulations that can be used in the training population.

Fig. 1 illustrates the data structure of a training population 44 in accordance with one embodiment of the present invention. There is a record 46 for each subject in training
10 population 44. Each record 46 includes an optional subject identifier 48 for uniquely identifying the subject. Each record 46 includes a molecular marker data file 50 for storage of the molecular marker data measured in step 202. Fig. 4 provides more details on a molecular profile 50 resulting from Molecular Marker data processing module I in accordance with one embodiment of the present invention. The molecular profile 50 of
15 Fig. 4 includes an identifier 302 for each molecular marker 302 tracked by profile 50. Then, for each respective molecular marker 302 in profile 50, there exists one or more measurements of molecular marker data 304. In some embodiments, more than one data point is measured for molecular markers 302. If more than one data point is measured for a molecular marker, then a statistical measure of central tendency (e.g. mean, median,
20 average etc.) can be computed. Accordingly, such measurements for molecular marker data 304 can be stored in data structure 50.

There exists a trait characterization field 52 for each subject in training population 44. Preferably as many as possible traits of each member of training population 44 are documented in a trait characterization record (52). Documented traits include known
25 condition or clinically measurable parameters; genetic likelihood of disease or condition; medications both past and current; environmental exposures, ethnicity, age, sex and the like. In some embodiments, training population 44 includes only two trait subgroups and trait characterization 52 is a binary choice between two values, where one value indicates that the corresponding subject belongs in a first trait subgroup and a second value indicates
30 that the subject belongs in a second trait subgroup. In some embodiments, training population 44 is divided into a plurality of lists, where each list in the plurality of lists represents a different trait subgroup. In such embodiments, there is no need for a phenotypic characterization field 52.

Although not illustrated in Fig. 1, in some embodiments, there exists a scoring population in addition to the training population. The scoring population is used to evaluate each of the classifiers derived from the training population. The scoring population is made up of one or more individuals that have at least two trait subgroups in common with the training population. In some embodiments, multiple scoring populations are generated from the training population using one or more resampling or cross validation procedures including: bootstrapping; leave one out; leave n out; percent split and the like so as to evaluate the classifiers derived from the training population. In preferred embodiments, the members of the scoring population are not the members used in the training population.

In some embodiments, some aspects of step 202 are performed by first data molecular processing module 54. As such, first data molecular processing module 54 can be a known software program, such as commercially available and/or academically available data processing programs.

Step 204.

In step 204, using the data measured in step 202, individual candidate molecular markers are identified, where the molecular marker data allows the differentiation as between two of the trait subgroups of the training population. In some embodiments, step 204 represents a series of pairwise comparisons, where each pairwise comparison is between molecular marker data for subjects from two different trait subgroups. In other words, data associated with molecular markers from a population of samples having one aspect of a trait of interest (a first trait subgroup) are compared with a population of samples having a second aspect of a trait of interest (a second trait subgroup) so as to identify molecular markers that are able to differentiate between the two trait subgroups (ie the molecular marker data enables the ability to differentiate between the two trait subgroups).

In instances where more than two trait subgroups are represented by a training population 44, more than two pairwise comparisons can be performed on the molecular marker data to identify lists of candidate molecular markers for each of the possible pairwise comparison.

A number of statistical techniques can be used to perform the pairwise comparisons of step 204. In some embodiments, standard statistical techniques such as a t-test are used. Methods based on conventional t-tests provide the probability (P) that a difference in measured values for the data of a molecular marker between two different trait subgroups

occurs by chance. See, for example, Baldi *et al*, 2001, Bioinformatics 17, pp. 509-519, 2001, which is hereby incorporated herein by reference in its entirety. The t-test compares the actual difference between two means in relation to the variation in the data (expressed as the standard deviation of the difference between the means). For instance, to determine
5 whether a particular molecular marker discriminates between a first trait subgroup and a second trait subgroup, the mean of the data for the molecular marker in the first trait subgroup is compared to the mean of the data for the molecular marker in the second subgroup in accordance with the t-test. In some embodiments, the molecular marker data is such that it is deemed to discriminate between two trait subgroups when the t-test yields
10 a score that matches or exceeds the $p = 0.05$ level (95% confidence, "significant confidence"), the $p = 0.01$ level (99% confidence, "highly significant confidence") or $p = 0.001$ (99.9% confidence, "very highly significant confidence"). In some embodiments, the t-test is applied with a Bonferroni correction or similar form of correction, in some
15 embodiments, rather than using a t-test, nonparametric equivalents such as the Wald-Wolfowitz runs test, the Mann-Whitney U test, the Kolmogorov-Smirnov two-sample test or ROC are used.

In some embodiments, training population molecular marker data is obtained using a microarray and the Significant Analysis of Microarrays technique of Tusher *et al*. is used to identify molecular markers whose data discriminates between trait subgroups. See, for
20 example, Tusher *et al*, 2001, Proc. Natl. Acad. Sci. USA 98, 5116-5121. In some embodiments, Manduchi's algorithm for assigning confidence to differentially expressed genes is used to identify molecular markers whose data discriminates trait subgroups. See, for example, Manduchi *et al*, 2000, Bioinformatics 16, 685-598.

In some embodiments, there are a number of different trait subgroups represented
25 within the training population. Thus, in such embodiments, application of a series of pairwise t-test can become computationally intensive and prone to underinclusiveness in the identification of discriminating molecular markers resulting for each binary comparison since the number of pairwise comparisons that must be performed grows quickly as a function of the number of trait subgroups present in training population 44.
30 For example, if there are seven trait subgroups present in training population 44, a total of 21 pairwise (t-test class) test can be performed. In such embodiments, analysis of variance (ANOVA) can be used to simultaneously consider whether the data for a molecular marker produces statistically different means for each of the phenotypic data structures. ANOVA considers the data for a given molecular marker from each of the trait subgroups present in

training population 44 and produces a single number (the F-statistic) that can be evaluated for significance at any desired confidence value (e.g., the $\alpha = 0.05$ level, the $\alpha = 0.01$ level, the $\alpha = 0.001$ level, etc). ANOVA is described, for example, in Draghici, *Data Analysis Tools For DNA Microarrays*, 2003, Chapman & Hall, CRC Press, New York, pp. 155-187, which is hereby incorporated herein by reference in its entirety. In some embodiments, nonparametric equivalents to ANOVA, such as the *Kruskal-Wallis analysis of ranks* test, the Median test, Friedman's two-way analysis of variance, or the Cochran Q test, are used to identify molecular markers that have statistically different means as between two of the trait subgroups. In some embodiments, after running ANOVA, a means comparisons test such as Duncan's, Student-Newman-Keuls (SNK), Tukey-Kramer, Tukey's HSD, or Least Significant Difference (LSD) are run to determine which molecular markers have data that statistically differentiates as between one or more of the trait subgroups tested (e.g. has statistically different values in one or more of the trait subgroups tested). In some embodiments, such tests are performed instead of ANOVA or pairwise *t*-tests to identify molecular markers that discriminate two or more trait subgroups.

In some embodiments, a parametric test and a nonparametric test are run in step 204. If there are only two trait subgroups being compared (e.g., non-disease versus disease) then a Welch *t*-test (parametric test) and a Mann-Whitney test are used without multiple test corrections at decreasing *p*-values (e.g., $p < 0.05$, $p < 0.01$, $p < 0.005$, $p < 0.001$, etc.) until the number of candidate molecular markers is less than 50, less than 100, less than 150, less than 200, less than 250 etc.. If three or more trait subgroups are being compared, the Kruskal-Wallis (nonparametric) and Welch ANOVA (parametric) tests are used. Following the same procedure for instances where only two trait subgroups are being compared, additional molecular marker sets are produced in conjunction with multiple test corrections. An example of a test correction is the Benjamin-Hochberg false discovery rate. In some embodiments the post-hoc statistical tests for groups of three or more provided in GeneSpring version 6.1+ is used. The post-hoc test provides a means for determining which molecular markers are different between particular trait subgroups. Such post-hoc tests include the Student Newman Keuls test and the Tukey test.

In some embodiments, data for a test molecular marker from all possible trait subgroups in training population 44 is not used. Rather, similar to the case where pairwise *t*-tests are used, ANOVA is used to determine whether the data for a molecular marker can discriminate between some or all of the trait subgroups. For example, consider the case in which training population 44 includes five trait subgroups. In one approach, a pairwise *t*-

test can be used to identify molecular markers that statistically discriminate (e.g. $p = 0.05$) between one of the possible pairs of trait subgroups in the training population. Similarly, ANOVA can be used to identify molecular markers that statistically discriminate between one of the possible triplets in the training population. Alternatively, ANOVA can be used to identify molecular markers that statistically discriminate between one of the possible quadruplets in the training population.

In some embodiments, either in addition to using statistical methods, or independently of statistical methods such as described herein, processing step 204 to select candidate molecular markers is done by looks for differential data abundances (e.g., differential expression). Such methods differ from those described above in the sense that variance as between the level of expression as between members of the same trait subgroup are not necessarily considered. In order to compare differential expression as between two or more trait subgroups, a statistical measure of central tendency (e.g. mean, median, average etc.) can be computed for each molecular marker within any trait subgroup. In some embodiments, the measure of differential data abundance (or differential expression) for a molecular marker product as between a first trait subgroup and a second trait subgroup is determined by measuring the fold change, for example a fold change of greater than 1.5, 2.0, 2.5, 3.0, 4.0 or higher can be selected.. In another embodiment, the measure of differential data abundance need not be quantified, but molecular markers products which on visual inspection display a clear difference in expression (ie abundance levels) as between two trait subgroups can be selected. To illustrate, consider a population P' that comprises trait subgroups "A" and "B". That is, each member of P' is classified into either subgroup "A" or "B" based on whether or not they exhibit or have a particular trait. In this situation, products of molecular markers that are present in large quantities/abundance in one group ("A" or "B") but not the other group are identified. For instance, molecular markers that strongly express in subgroup "A" but not in subgroup "B" can be identified from the measurements taken for each member of each subgroup in step 202. Likewise, molecular markers that express strongly in subgroup "B" but not subgroup "A" can be identified. These patterns of differential abundance of the products of the molecular markers can be used to identify candidate molecular markers. For an illustration of this approach, see Glob et al. 1999, Science 286: 531.

In embodiments where molecular markers are identified in select pairwise tests, such as pairwise *t*-tests or ANOVA tests of subsets of the total number of trait subgroups in training population 44, there might be several different lists of molecular markers. For

example, molecular marker list A might include molecular markers whose measured data (including normalized data etc.) discriminates between a first and second trait subgroup as determined by a first pairwise *t*-test. Molecular marker list B might include molecular markers whose data discriminates between a first and third trait subgroup as determined by a second pairwise *t*-test. Molecular marker list C might include molecular markers whose data discriminates between a first, second and third trait subgroup as determined by ANOVA. In some embodiments, each candidate molecular marker list 60 is preserved as an independent list in candidate molecular marker data structure 58 (Fig. 1).

In some embodiments, processing step 204 identifies a total of between 10 and 4000 candidate molecular markers for any particular training population. In other embodiments, step 204 identifies a total of between 500 and 2000 candidate molecular markers. In yet another embodiment, processing step 204 identified a total of between 100 and 1000 candidate molecular markers.

In some embodiments, some aspects of step 204 are performed by molecular marker candidate identification module 56A. Additionally, there exist known programs that can perform some of the functionality described in step 204. Such programs include those formerly sold by Silicon Genetics (e.g. GeneSpring™); now Agilent Technologies.

In some embodiments, candidate molecular markers identified using the above-described statistical or nonstatistical tests are clustered in order to visualize relationships between the genes. For example, in some embodiments GeneSpring™ is used to perform hierarchical clustering using the Spearman correlation statistic. In some embodiments, QT clustering is used to identify genes that have a similar pattern of expression across the specimens in training population 44. Clustering that can be employed in step 204 is described generally in Section 5.12. Further, Section 5.12 gives examples of some clustering techniques that can be used in step 204. Molecular markers for use in subsequent sections (e.g., for quantitative measurement using methods as described in step 206 below) can be ranked and selected by one or more criteria, including, but not limited to, fold change differences in molecular marker data between two or more trait subgroups, standard deviations of molecular marker data as between two or more trait subgroups using the above-described statistical tests, coefficient of variation, statistical significance (e.g., *p*-value from ANOVA and/or *Mest*, or other tests described above), level of expression as determined using molecular marker data, gene function, reproducibility of molecular marker data (includes intra- and inter-experimental), elucidated pathways / networks of the molecular markers as would be understood by a person skilled in the art (e.g. selecting a

molecular marker on the basis of an understanding of how said gene is known to function in the body) and the like. Thus in some embodiments, molecular markers for use in subsequent sections are chosen on the basis of the p value identified as a result of step 204 as a measure of the likelihood that the molecular marker data can distinguish as between

5 the two trait subgroups and more particularly molecular markers are chosen wherein the p value is less than 0.5; less than 0.1, less than 0.05, less than 0.01, less than 0.005, less than 0.001, less than 0.0005, less than 0.0001, less than 0.00005, less than 0.00001, less than 0.000005, less than 0.000001 etc. In some embodiments, molecular markers for subsequent steps are chosen on the basis of the level of differential expression displayed

10 by the molecular marker products as between the two or more trait subgroups. Note that in measuring differential fold change in blood, the fold change differences can be quite small, thus in some embodiments, selection of molecular markers is based on a differential fold change where the fold change is greater than 1.2, greater than 1.3, greater than 1.4, greater than 1.5, greater than 1.6, greater than 1.7, greater than 1.8, greater than 1.9, greater than

15 2.0, greater than 2.1, greater than 2.2, greater than 2.3, greater than 2.4, greater than 2.5, greater than 2.6, greater than 2.7, greater than 2.8, greater than 2.9, greater than 3.0, greater than 3.1, greater than 3.2, greater than 3.3, greater than 3.4 greater than 3.5, greater than 4.0 and the like. In some embodiments, it is helpful to select molecular markers on a basis of the combination of both p value and fold change as would be understood by a person

20 skilled in the art. Thus in some embodiments, molecular markers are first selected as outlined above on the basis of the p value resulting from the molecular marker data and then a subselection of said molecular markers is chosen on the basis of the differential fold change determined from the molecular marker data. In other embodiments, molecular markers are first selected on the basis of differential fold change, and then subselection is

25 made on the basis of p value, In some embodiments, the use of one or more of the selection criteria and subsequent ranking permits the selection of the top 2.5%, 5%, 7.5%, 10%, 12.5%, 15%, 17.5%, 20%, 30%, 40%, 50% or more of the ranked molecular markers for use in subsequent steps. In other embodiments, the selection criteria noted above can be set on the basis of the desired number of selected molecular markers for use in steps

30 206 and or other steps leading to the selection of the available set of markers for step 214. As would be understood, a selection criteria based on the desired number of selected molecular markers will depend upon the resources available for obtaining the molecular marker data for step 206 and/or the computer resources available for calculating and evaluating classifiers of all or a portion of possible combinations of the selected molecular

markers. In some embodiments, the desired number of selected molecular markers for use in step 214 can be 4,000; 3,000; 2,000; 1,000; 900; 800; 700; 600; 500; 400; 300; 200; 190; 180; 170; 160; 150; 140; 130; 120; 110; 100; 90; 80; 70; 60; 50; 40; 30; 20; 10. The more molecular markers which can be selected for use in step 214; the greater the

5 likelihood of identifying classifier or classifiers which are particularly useful for diagnosis.

In some embodiments, one or more subjects of the training population are identified as outliers and are removed prior to identifying individual candidate molecular markers as described herein. These outlier members can then be removed from the training population prior to proceeding to later steps. As described herein, in one

10 embodiment a neural network is used to identify such outliers. A neural network has a layered structure that includes, at a minimum, a layer of input units (and the bias) connected by a layer of weights to a layer of output units. Such units are also referred to as neurons. For output along a single dimension, the layer of output units includes just one output unit. However, neural networks can handle multiple quantitative responses (outputs

15 along multiple dimensions) in a seamless fashion by providing multiple units in the layer of output units.

In multilayer neural networks, there are input units (input layer), hidden units (hidden layer), and output units (output layer). There is, furthermore, a single bias unit that is connected to each unit other than the input units. Neural networks are described in

20 Duda *et al*, 2001, *Pattern Classification*, Second Edition, John Wiley & Sons, Inc., New York; and Hastie *et al*, 2001, *The Elements of Statistical Learning*, Springer-Verlag, New York.

The basic approach to the use of neural networks to identify outliers is to start with an untrained network. A training pattern is then presented to the untrained network. This

25 training pattern comprises a training population and, for each respective member of the training population, an association of the respective member with a specific trait subgroup. Thus, the training pattern specifies measured molecular marker data from blood for molecular markers for each member of a training population as well as an indication as to which trait subgroup each member of the training population belongs. In preferred

30 embodiments, training of the neural network is best achieved when the training population includes members from more than one trait subgroup.

In the training process, individual weights in the neural network are seeded with arbitrary weights and then the molecular marker data for each member of the training population is applied to the input layer. Signals are passed through the neural network and

the output determined. The output is used to adjust individual weights. A neural network trained in this fashion classifies each individual of the training population with respect to one of the input trait subgroups. In typical instances, the initial neural network does not correctly classify each member of the training population. Those individuals in the

5 training population that are misclassified identify and determine an error or criterion function for the initial neural network. This error or criterion function is some scalar function of the trained neural network weights and is minimized when the network outputs match the desired outputs. In other words, the error or criterion function is minimized when the network correctly classifies each member of the training population into the

10 correct trait subgroup. Thus, as part of the training process, the neural network weights are adjusted to reduce this measure of error. For regression, this error can be sum-of-squared errors. For classification, this error can be either squared error or cross-entropy (deviation). See, *e.g.* Hastie *et al.*, 2001, *The Elements of Statistical Learning*, Springer-Verlag, New York. Those individuals of the training population which are still incorrectly

15 classified by the trained neural network, once training of the network has been completed, are identified as outliers and can be removed prior to proceeding.

In some embodiments, an ensemble of neural networks can be used on the training population and individuals ranked on the basis of the number of times an individual is misclassified by each neural network. In order to create the ensemble, each neural network

20 can differ with respect to the initial seeded weighting. In another embodiment, each neural network can differ on the basis of randomly generated noise added to the molecular marker data for one or more molecular markers of each individual of the training population added to the input layer. In such an embodiment, this randomly generated noise can be applied by changing the amount of each of the measured molecular marker data of each member of

25 the training population by a scaled random amount. When larger amounts of noise are required, the magnitude of the scaled random amount is increased. In any of these embodiments, the number of layers and the number of units in each layer can be adjusted in order to provide optimal results for any given set of conditions. In this manner, one can identify outliers which are misclassified in as many as 0%, 20%, 30%, 40%, 50%, 60%,

30 70%, 80%, 90% or more of the neural networks used.

Step 205.

Step 205 is optional. We have surprisingly found that certain molecular markers whose molecular marker data fails to discriminate individually as between two trait subgroups in step 204, are still more than incrementally useful when utilized in

combinations with other candidate molecular markers selected in step 204. In particular we have been able to identify molecular markers whose data fails to individually discriminate as between two trait subgroups in the pairwise comparison of step 204 but contribute to a classifier identified in step 216 from a combination which includes one or
 5 more candidate molecular markers identified in step 204 ("combination friendly molecular markers"). Thus, in order to ensure that molecular markers which may be useful in combinations are not removed prematurely, optional step 205 is performed.

In step 205, all or a portion of the molecular markers for which data has been or can be obtained in at least two of the trait subgroups of the training population are utilized
 10 ("putative combination molecular markers"). For purposes of step 205, in one embodiment, the data is obtained using a technique which allows for fast and efficient data generation for all of the molecular markers of the genome of interest chosen. In another embodiment the data is obtained using one or more of the techniques as described in Section 5.3 and/or 5.4. In another embodiment, the data is obtained using microarray
 15 technology. In a preferred embodiment, the data used is the data obtained for step 202

In order to identify additional candidate molecular markers, combinations of molecular markers are chosen and a mathematical model applied to the molecular marker data for each molecular marker of the combination resulting in a classifier for each combination. The mathematical model applied can be selected from those defined in
 20 Section 5.14. In some embodiments, each possible combination of 2, and/or 3, and/or 4, and/or 5, and/or 6, and/or 7, and/or 8, and/or 9 and/or 10 or more of the putative combination friendly molecular markers are tested. For example, if there are 8,000 putative combination friendly molecular markers, each possible combination of 8 or less molecular markers can be written as follows:

25
$$\frac{8,000!}{((8,000 - 8)! 1 (8)!)}$$

each classifier resulting from each combination is scored as described more fully in step 220. In some embodiments, the classifiers are scored using the training population so as to permit time and cost savings. In other embodiments, the classifiers are scored using a scoring population. In yet other embodiments, the classifiers are scored using other
 30 resampling or cross validation procedures so as to generate multiple scoring populations. Having scored the classifiers, a subset of classifiers are then selected based on the score.

In some embodiments, the subset of classifiers is any number less than the total number of combinations evaluated. In some embodiments, the top 10%, top 20%, top 30%, top 40%, or top 50% of the classifiers generated are chosen. In some embodiments,

wherein scoring is done using ROC area under the curve, those classifiers with an ROC area under the curve of 0.5, 0.6, 0.7, 0.8, 0.9 or 1.0 are selected.

Having selected a number of classifiers, each representing a combination of molecular markers, the number of occurrences of each putative combination friendly molecular marker in the combinations of the selected classifiers are determined. Putative combination friendly molecular markers can then be selected as combination friendly molecular markers so as to used as candidate molecular markers based on the number of reoccurrences of said molecular marker in the selection of combinations evaluated. In one embodiment, 5, 10, 15, 20, 30, 50, 100, 150, 200 or more additional candidate molecular markers not previously selected in step 204 are chosen to proceed to step 206. In other embodiments, the top 10%, top 20%, top 30%, top 40%, or top 50% of combination friendly molecular markers as determined by reoccurrence statistics are selected to be included in the selected set of candidate molecular markers for purposes of choosing combinations to create classifiers in accordance with steps 214 to 218.

15 *Step 206.*

In step 206, second molecular marker data is obtained for the candidate molecular markers identified in step 204. In one embodiment, the second molecular marker data is measured using any technique described in Section 5.3 or 5.4 or equivalents thereof. In another embodiment, the same technique used to measure the first molecular marker data in step 202 is used to obtain the second molecular marker data. In other embodiments, an alternative technique is used to measure the second molecular marker data. In other embodiments, the first molecular marker data is obtained using one of the following techniques: microarray, and/or RT-PCR and the second molecular marker data is obtained using any technique but microarray. In other embodiments, the first molecular marker data is obtained using microarray and the second molecular marker data is obtained using any technique except for microarray. The use of second molecular marker data is preferred because the changes in differential expression or abundance of the product of the molecular marker in blood as between trait subgroups can be as low as a 1.1 fold, 1.2 fold, 1.3 fold, 1.4 fold, 1.5 fold etc. which makes less sensitive and reproducible techniques less reliable. In addition, techniques which are preferable for the data collection to allow large scale screening in step 202 such as microarray have been shown to have significant inherent reproducibility issues with high standard deviation as between experiments. As such it is necessary to obtain second molecular marker data so as to ensure the accuracy of the ultimate classifiers identified.

Techniques utilized to obtaining second molecular marker data for the plurality of molecular marker products are those techniques known to measure abundance of RNA and/or protein including the techniques described in Section 5.3 and 5.4.

5 In some embodiments, it is helpful to obtain a third series of molecular marker data (ie third molecular marker data, fourth molecular marker data etc.) which can be molecular marker data of the training population used in steps 202 or of a different training or scoring population using any known technique including those techniques described in sections 5.3 and 5.4. Preferably more expensive and/or time consuming techniques are used once smaller numbers of candidate molecular markers have been identified

10 In some embodiments, some aspects of step 206 are performed by molecular marker data processing module 11 - 61. The exact nature of the functionality of molecular processing module 61 will depend on the type of measurement assay used in step 206. However, it is contemplated that module 61 will be used to record measurement values for molecular marker data in a profile similar to molecular marker data processing module 1 - 15 50, perform any necessary error correction techniques, normalization techniques (*e.g.*, techniques described in Bevington and Robinson, *Data Reduction and Error Analysis for the Physical Sciences*, Second Edition, WCB/ McGraw-Hill, 1992, *etc.*) and/or perform any measurement techniques that can be coded in a digital computer .

Step 208.

20 Step 208 is optional and allows for the selection of individual candidate molecular markers of step 206 which can be removed prior to the process of selecting and evaluating combinations of molecular markers in steps 214/216. In optional step 208, one or more candidate molecular markers in data structure 58 (Fig. 1) are eliminated. In optional step 208, the same types of tests that were performed in step 204 can be performed. The main 25 difference is that in step 208, the quantitative data measured in step 206 using low throughput methods is used whereas in step 204, the high throughput data measured in step 202 is used. Data measured in step 206 is used in step 208 to validate the candidate molecular markers.

30 In one specific embodiment, training population 44 consists of a first trait subgroup and a second trait subgroup and step 208 comprises performing a t-test or a nonparametric equivalent of the t-test on each candidate molecular marker using the molecular marker data measured in step 206 to verify for each candidate molecular marker that the molecular marker data differentiates between the first trait subgroup and the second trait subgroup with some measure of statistical confidence. Candidate molecular markers whose

molecular marker data are less effective in differentiating between the two trait subgroups (e.g. have a p value that is greater than .05) are removed from data structure 58 and are no longer considered as candidate molecular markers.

In another specific embodiment, training population 44 consists of a first trait subgroup, a second trait subgroup, and a third trait subgroup. In this specific embodiment, ANOVA or a nonparametric equivalent is performed independently on each candidate molecular marker in data structure 58 to verify that each molecular marker differentiates between the three subgroups using the molecular marker data. Candidate molecular markers whose molecular marker data are less effective in differentiating between the three trait subgroups (e.g. have a p value that is greater than .05) are removed from data structure 58 and are no longer considered as candidate molecular markers.

In some embodiments, each molecular marker is validated by using the data for each molecular marker to generate a Receiver Operating Characteristic (ROC) curve.

ROC curves are generally discussed in Park et al., Korean J. Radiol. 5, p. 11, which is hereby incorporated herein by reference in its entirety. In one embodiment of the present invention, an ROC curve is computed for each candidate molecular marker in training population 44 using the molecular marker data measured in step 206. As noted in step 202, training population 44 includes, for each specimen in the training population, an indication 52 as to whether or not the specimen has a particular trait under study.

Each respective ROC curve graphs the True Positive Fraction (TPF) as compared with 1/ the False Positive Fraction (FPF). For example, consider the case in which molecular marker A is being validated and the data for molecular marker A that was measured in step 206 is expression level of the molecular marker A in blood samples from subjects. Table B provides hypothetical values for the abundance of A across training population 44.

Table B: Values for molecular marker data of A across training set 44.

[A]	Presence / Absence of Disease
453	Y
437	Y
424	Y
374	Y
202	N
158	Y
102	N
37	N
0.54	N

- In Table B, each line represents a different specimen in the training population. If the relationship between [A] (data of cellular constituent A) and the presence of disease in subjects in the training population is statistically very significant, all positive results (where specimens have the disease) would be at the top of Table B and all negative results (where biological specimens do not have the disease) at the bottom of the Table B.

To plot the ROC curve corresponding to the test illustrated in Table B, the table is divided into a number of cutoff levels. Then, the sensitivity (TPF) and specificity (TNF) of each cutoff level is computed. Sensitivity and specificity are defined with reference to the decision matrix of Table C.

Table C: Decision matrix.

Test result	True Feature Status		
	Positive	Negative	Total
Positive	TP	FP	T+
Negative	FN	TN	T-
Total	D+	D-	

- In Table C, TP means the number of true positives, FP means the number of false positives, FN means the number of false negatives, and TN means the number of true negatives.

Sensitivity is the proportion of subject with a trait (*e.g.*, a disease or particular biological phenotype) who test positive for the feature. In probability notation sensitivity is $P(T^+|D^+) = TP / (TP + FN)$. Specificity is the proportion of patients without the trait who test negative for the feature. In probability notation specificity is $P(T^-|D^-) = TN / (TN + FP)$.

The ROC curve is defined as a plot of the sensitivity as the y-coordinate versus 1-specificity (false positive rate) as the x-coordinate. Thus, for Table B, where each line of the Table B represents an independent cutoff level, the following ROC data points are derived.

Table D: ROC data points for Table B.

Ratio Cutoff Level	Sensitivity	1-Specificity
No row	0	0
First row	0.2	0
First two rows	0.4	0
First three rows	0.6	0
First four rows	0.8	0

Ratio Cutoff Level	Sensitivity	1-Specificity
First five rows	0.8	0.25
First six rows	1	0.25
First seven rows	1	0.5
First eight rows	1	0.75
First nine rows	1	1

To compute the last row of Table D, the number of TP, FP, FN, and TN are counted in Table B when the condition is imposed that the classifier predicts that no specimen in Table B is positive for presence of the trait (e.g., disease or a particular biological phenotype). This, of course, is not an accurate classifier as reflected in the respective sensitivity and specificity values of 0 and 1. Plotting sensitivity by 1-specificity yields the coordinate (0,0) as illustrated in the last row of Table D. Figure 7 illustrates the ROC curve based upon the data points illustrated in Table D. As illustrated in Fig. 7, a ROC curve begins at coordinate (0,0) and ends at coordinate (1,1).

Once an ROC curve has been computed for a molecular marker, in one embodiment, the area under the ROC curve can be quantified. Generally, an area of 1.0 represents a molecular marker that is a perfect diagnostic indicator of the presence of absence of the trait. Preferably an area of greater than 0.5 is desired for a diagnostic indicator, but it will depend upon the trait of interest. For example measurement of protein PSA levels currently used to diagnose prostate cancer has an ROC of 0.47.

In some embodiments there are as many as fifty candidate molecular markers in data structure 58 at this stage of the inventive method. In some embodiments there are more than fifty candidate molecular markers. In practice, the number of candidate molecular markers that remain can be set to any desired number by raising or lowering the criteria for eliminating molecular markers. For example, smaller p values from ANOVA or t-tests, or larger ROC curve areas can be required if the total number of molecular markers is too large.

Steps 210 and 212.

Step 210 is optional and allows the additional removal of individual candidate molecular markers of step 206 by evaluating how each individual candidate molecular marker performs within a model which evaluates a combination of molecular markers prior to performing the evaluation of combinations in step 214/216.

In optional step 210 all or a portion of the remaining candidate molecular markers in data structure 58 are used to generate a regression classifier. To compute the regression classifier, measured data from step 206 for the molecular markers in two different trait

subgroups in training population 44 are used. In some embodiments, the two different trait subgroups respectively represent a diseased and nondiseased state. In some embodiments, the two different trait subgroups respectively represent a first diseased state (e.g. cancer) and a second unrelated diseased state (e.g., Alzheimer's disease). In some embodiments, the two different trait subgroups represent those subjects that are responsive to drug therapy and those subjects that are not responsive to drug therapy. In still other embodiments, the two different trait subgroups from which molecular marker data is obtained represent data from subjects obtained *a priori* to treatment, but that have been classified into different trait subgroups on the basis of the ultimate response to treatment.

10 In some embodiments, the two different trait subgroups respectfully represent two different stages of a disease (e.g., moderate versus advanced).

In some embodiments, data for between ten and thirty candidate molecular markers in the two select trait subgroups is used in the logistic regression. In some embodiments, between twenty and one hundred candidate molecular makers in the two select trait subgroups is used in the logistic regression. In still other embodiments, all the candidate molecular markers in the two select trait subgroups are used in the logistic regression.

In step 210 logistic regression can be used because one of the dependent variables is binary - absence or presence of a particular phenotype. For example, consider the case in which molecular marker data from a first trait subgroup and molecular marker data from a second trait subgroup is used in step 210. The first trait subgroup is characterized by a first disease and the second trait subgroup is characterized by a second disease. In such instances, what can be considered by logistic regression is absence or presence of the first disease in subjects. Alternatively, what can be considered by logistic regression is absence or presence of the second disease in subjects.

25 In general, the multiple regression equation of interest can be written

$$Y = a + \beta_1 X_1 + \beta_2 X_2 + \dots + \beta_k X_k + \varepsilon$$

where Y , the dependent variable, is presence (when Y is positive) or absence (when Y is negative) of the trait (e.g., phenotype, condition) associated with the first trait subgroup considered in step 204. This classifier says that the dependent variable Y depends on k explanatory variables (the measured data values for the k candidate molecular markers from subjects in the first and second trait subgroups in training population 44), plus an error term that encompasses various unspecified omitted factors. In the above-identified classifier, the parameter β_1 gauges the effect of the first explanatory variable X_1 on the

dependent variable Y , holding the other explanatory variables constant. Similarly, β_2 gives the effect of the explanatory variable X_2 on Y , holding the remaining explanatory variables constant.

In general, in the multiple regression procedure, estimates for β_1 are obtained by taking into account how uncontrolled changes in other variables influence Y . Thus, in specific embodiments of the present invention, regression is used to eliminate at least some of the candidate molecular markers rather than relying entirely on the tests described in step 208 because the regression takes into account patterns in which multiple molecular markers influence the dependent variable (absence or presence of a trait) in a concerted fashion.

Because the dependent variable data is binary, logistic regression can be used. The logistic regression classifier is a non-linear transformation of the linear regression. The logistic regression classifier is termed the "logit" classifier and can be expressed as

$$\ln[p/(1-p)] = \alpha + \beta_1 X_1 + \beta_2 X_2 + \dots + \beta_k X_k + \epsilon \quad \text{or}$$

$$[p/(1-p)] = \exp^{\alpha} \exp^{\beta_1 X_1} \exp^{\beta_2 X_2} \dots \exp^{\beta_k X_k} \exp^{\epsilon}$$

where,

\ln is the natural logarithm, \log_e , where $e=2.71828\dots$,
 p is the probability that the event Y occurs, $p(Y=1)$,
 $(1-p)$, the probability that the event Y does not occur, $p(Y \neq 0)$,
 $p/(1-p)$ is the "odds ratio",
 $\ln[p/(1-p)]$ is the log odds ratio, or "logit", and
 all other components of the classifier are the same as the general regression equation described above. It will be appreciated by those of skill in the art that the term for α and ϵ can be folded into a single constant. Indeed, in preferred embodiments, a single term is used to represent α and ϵ . The "logistic" distribution is an S-shaped distribution function. The logit distribution constrains the estimated probabilities (p) to lie between 0 and 1.

In some embodiments of the present invention, the logistic regression classifier is fit by maximum likelihood estimation (MLE). In other words, the coefficients {e.g., α , β_1 , β_2 , ...} are determined by maximum likelihood. A likelihood is a conditional probability (e.g., $P(Y|X)$, the probability of Y given X). The likelihood function (L) measures the

probability of observing the particular set of dependent variable values (Y_1, Y_2, \dots, Y_n) that occur in the sample data set. It is written as the probability of the product of the dependent variables:

$$L = \text{Prob}(Y_1 * Y_2 *** Y_n)$$

- 5 The higher the likelihood function, the higher the probability of observing the Y s in the sample. MLE involves finding the coefficients ($\alpha, \beta_1, \beta_2, \dots$) that makes the log of the likelihood function ($LL < 0$) as large as possible or -2 times the log of the likelihood function (-2LL) as small as possible. In MLE, some initial estimates of the parameters $\alpha, \beta_1, \beta_2, \dots$ are made. Then the likelihood of the data given these parameter estimates is
- 10 computed. The parameter estimates are improved the likelihood of the data is recalculated. This process is repeated until the parameter estimates do not change much (for example, a change of less than .01 or .001 in the probability). Examples of logistic regression and fitting logistic regression classifiers are found in Hastie, *The Elements of Statistical Learning*, Springer, New York, 2001, pp. 95-100, which is hereby incorporated by
- 15 reference in its entirety.

Step 212.

- In specific embodiments, all or a portion of the candidate molecular markers are used and the molecular marker data fit using logistic regression. Then, in a stepwise fashion, some of the molecular markers are eliminated from the classifier using backward
- 20 stepwise regression. Backward stepwise regression begins with a full or saturated classifier and variables are eliminated from the classifier in an iterative process. The fit of the classifier is tested after the elimination of each variable (molecular marker) to ensure that the classifier still adequately fits the molecular marker data. When no more variables can be eliminated from the classifier or a desired number of molecular markers remain in
- 25 the classifier, the analysis has been completed. In specific embodiments, the regression applied in step 210 is used to refine the candidate molecular marker list to less than 25, less than 24, less than 23, less than 22, less than 21, or less than 20 molecular markers.

- In one embodiment, a logistic regression classifier is computed using all or a portion of the available candidate molecular markers in data structure 58. Then,
- 30 coefficients are tested for significance for inclusion or elimination from the classifier using a Wald test, a likelihood-ratio test (chi-squared statistic), a Hosmer-Lemshow Goodness of Fit Test, or the like. For example, the likelihood-ratio test uses the ratio of the maximized value of the likelihood function for the full classifier (L_1) over the maximized value of the

likelihood function for the simpler classifier (L_0) in which one or more molecular markers have been removed. The likelihood-ratio test statistic equals:

$$-2\log\left(\frac{L_0}{L_1}\right)$$

This log transformation of the likelihood functions yields a chi-squared statistic.

5 *Step 213.*

Step 213 is optional. We have found that performing optional step 213 provides a significant improvement in identifying classifiers which are particularly useful in diagnosis of a disease or condition of interest. In optional step 213, clinically measurable parameters are identified which are thought to be relevant to the trait of interest for which a classifier
10 is desired. For example, where the trait of interest is prostate cancer, clinically measurable parameters chosen are those that are known or are shown to be relevant to the trait of interest. For example in one embodiment, clinically measurable parameters relevant to prostate cancer can include age of subject, level of prostate specific antigen (PSA); and volume of prostate. In yet another embodiment, where the trait of interest is osteoarthritis,
15 some relevant clinically measurable parameters are age and body mass index (BMI). The selected clinically measurable parameters are then included as part of the "selected set of candidate molecular markers" and are treated as molecular markers for the purpose of selecting combinations and developing classifiers in step 214 through 218 as described below.

20 In order to treat the clinically measurable parameter as a molecular marker for purposes of step 214 through 218, the clinically measurable parameter must have associated data. In some embodiments, where the clinically measurable parameter is one which has an associated value - for example age, blood glucose level, PSA level, blood pressure, body mass index, etc., the value can be treated as the molecular marker data for
25 purposes of steps 214 through 218. In some embodiments there is no value associated with the clinically measurable parameter, for example where the relevant clinical parameter is determinable but does not provide a value. In those cases, a value can be assigned to represent each aspect of the clinically measurable parameter. For example where the sex of a person is the clinically measurable parameter, a value of 1 can be
30 assigned to represent that the person is male, and a value of 0 can be assigned to represent that the person is female. As yet another example, where the relevant clinically measurable parameter is ethnicity, a different value can be assigned to each ethnicity (e.g.

1 Caucasian, 2 asian, 3 ashkenazi jew etc. and said value can be used as the molecular marker data associated with ethnicity for purposes of step 214 through 218.

Step 214.

Steps 214 through 218 provide an approach in which all or a portion of the possible combinations of the selected set of candidate molecular markers resulting from steps 202-213 are chosen. Molecular marker data from each candidate molecular marker in a elected combination is applied to a mathematical model as described more fully in Section 5.14. If there are N selected molecular markers at this stage then, in some embodiments, as many as 2N-1 different combinations can be selected and classifiers can be computed for each of these combinations. For example, consider the case in which three molecular markers are selected after any combination of steps 201 through 212 have been performed and logistic regression is used in step 216. In this case, the following 2^N-1 mathematical models can be used to form 2^N-1 corresponding classifiers:

$$\ln[p/(1-p)] = \alpha + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \epsilon$$

$$\ln[p/(1-p)] = \alpha + \beta_1 X_1 + \beta_2 X_2 + \epsilon,$$

$$\ln[p/(1-p)] = \alpha + \beta_1 X_1 + \beta_3 X_3 + \epsilon,$$

$$\ln[p/(1-p)] = \alpha + \beta_2 X_2 + \beta_3 X_3 + \epsilon,$$

$$\ln[p/(1-p)] = \alpha + \beta_1 X_1 + \epsilon,$$

$$\ln[p/(1-p)] = \alpha + \beta_2 X_2 + \epsilon, \text{ and}$$

$$\ln[p/(1-p)] = \alpha + \beta_3 X_3 + \epsilon.$$

In these mathematical models, α , β_1 , β_2 , ..., β_N represent coefficients that are regressed against molecular marker data whereas X_1 , X_2 , ..., X_N each represent a different RNA or protein (or more generally, a molecular marker) for which molecular marker data is available. In some embodiments any one of elements X_1 , X_2 , ..., X_N can represent a clinically measurable parameters. In a preferred embodiment for each combination chosen, at least one of the molecular markers of the series of X_1 , X_2 , ..., X_N does not represent a clinically measurable parameter. In some embodiments, additional interaction

terms are also considered, producing non linear behaviour and resulting in greater than or less than as 2^{N-1} different combinations. In some embodiments, additional interaction terms are also considered, producing non linear behaviour. For instance, in the example above, another mathematical model to which molecular marker data can be applied in order to form a classifier is:

$$\ln[p/(1-p)] = \alpha + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_2 X_3 + \epsilon$$

where the coefficient β_4 represents the interaction between molecular marker X_2 and X_3 . In such embodiments, more than 2^{N-1} "combinations" and thus more than 2^{N-1} classifiers are considered. In addition to the possibility of interaction terms, the present invention encompasses nonlinear variables. Examples of nonlinear variables include variables that are squared, squared rooted, or in fact, taken to any power. For instance, additional examples of mathematical models to which molecular marker data can be applied include:

$$\ln[p/(1-p)] = \alpha + \beta_2 (X_2)^2 + \beta_3 X_3 + \beta_4 X_2 X_3 + \epsilon$$

$$\ln[p/(1-p)] = \alpha + \beta_2 (X_2)^{1/2} + \beta_3 X_3 + \beta_4 X_2 X_3 + \epsilon$$

In some embodiments, a logarithmic or exponential function is applied to one or more of the variables. In some embodiments, ratios of molecular marker data can be used as a mathematical model. For example, consider the case in which regression is used to apply molecular marker data to the following equation in order to develop a classifier:

$$\ln[p/(1-p)] = \alpha + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \epsilon$$

Above, it was noted that X_1, X_2, \dots, X_N each represent the product of a different gene (or more generally, a molecular marker or molecular marker like element) for which molecular marker data is available. However, in the case where ratios are selected for use in mathematical models which are subsequently scored, each X_1, X_2, \dots, X_N can in fact represent a ratio of abundance and/or expression levels for two different molecular marker products e.g. RNA or proteins, or any other type of molecular marker. For example, X_1 can represent the ratio between molecular marker data measured in step 206 representative of gene (molecular marker) A and data for gene B in training population 44. Mixed forms of mathematical models are also possible. For example, some variables X can represent ratios between the molecular marker data of two molecular markers whereas other variables X can represent molecular marker data of discrete molecular markers as opposed to ratios of molecular marker data. In one specific embodiment a "ratio" of the RNA products of two molecular markers can be used and all or some of the possible combinations of said "ratios" can be utilized. For example, where the molecular marker

data is a measure of abundance of RNA is determined using quantitative RT-PCR, the measure of the expression level of gene (molecular marker) A and the measure of the expression level of gene B can be used as a single term. In some embodiments this is done by first determining the level of expression of each gene individually as compared with an internal housekeeping control such as β -actin:

$$\text{e.g. } \Delta Ct = Ct_{\text{gene A}} - Ct_{\beta\text{actin}}$$

where $Ct_{\text{gene A}}$ is the threshold cycle of amplification of GeneA and $Ct_{\beta\text{actin}}$ is the threshold cycle of amplification of the internal control β -actin. Similarly the level of expression of gene B is also determined in comparison with an internal housekeeping control

$$\text{e.g. } \Delta Ct = Ct_{\text{gene B}} - Ct_{\beta\text{actin}}$$

In order to combine the terms into a single term for purposes of creating a classifier using "ratios" of the two terms - the terms are combined as follows to form a single variable (e.g. X).

$$\Delta Ct = Ct_{\text{gene A}} - Ct_{\text{gene B}}$$

This is commonly described as the use of ratios given the logarithmic nature of the measure of Ct. Thus in some embodiments, the X_1, X_2, \dots, X_N of a classifier, each term represents the "ratio" of two molecular markers. In other embodiments the Ct scores are compared directly rather than compared with an internal control.

For example, consider the case in which the desire is to form a classifier that discriminates between a first trait subgroup and a second trait subgroup wherein each term of the classifier represents molecular marker data which is derived from a combination of molecular markers using ratios as outlined above (e.g. $\Delta Ct = Ct_{\text{gene A}} - Ct_{\text{gene B}}$). In such a case, each variable actually represents two molecular markers - the ratio of the molecular marker data for two molecular markers. Therefore in instances, for example, where 10 molecular markers have been identified by the funneling process of steps 202-210, 45 possible combinations of "ratios" of molecular markers can be formed

$$\text{e.g. } n! / (n-2)!2!$$

where n is the number of possible molecular markers (e.g. 10).

In some embodiments it is particularly useful to select molecular markers where the molecular marker data will work well in the form of a "ratio" thus, as part of step 214, in one embodiment, prior to selecting combinations of molecular markers, molecular markers whose molecular marker data can be combined as ratios are first identified.

The use of molecular marker data as variables in which the variable representing the product of the molecular marker is in the form of ratios or raised to some arbitrary power (*e.g.*, α , 2, N, *etc.*) is not limited to mathematical models based on regression. Such variables can be used in any of the mathematical models described herein (*e.g.*, neural networks). For example, consider the case in which the desire is to form a classifier that discriminates between a first trait subgroup and a second trait subgroup wherein each term of the classifier is a combination of molecular markers evaluated as a ratio. In step 214, in one embodiment, prior to selecting ratios of molecular markers, molecular markers which can be combined as ratios are first identified. Molecular markers which can be combined as ratios are those molecular markers wherein the ratio as between said molecular markers is a value which is not equal 1.0 (or in one embodiment wherein $\Delta Ct = Ct_{\text{gene A}} - Ct_{\text{gene B}}$ does not equal zero. In one embodiment, a first set of molecular markers and a second set of molecular markers are selected to create ratios such that the first set of molecular marker data demonstrates the molecular marker is upregulated in the first trait subgroup (relative to the second trait subgroup) in training population 44 and a second set of molecular marker data demonstrates that the molecular marker is downregulated in the first trait subgroup in training population 44 (relative to the second trait subgroup). Thus, for example an upregulated gene is one in which $\Delta Ct = Ct_{\text{gene B}} - Ct_{\text{pactin}} > 0$ and a downregulated gene is one in which $\Delta Ct = Ct_{\text{gene A}} - Ct_{\text{pactin}} < 0$,

Here, the term upregulated or downregulated generally means that such up or down regulation is observed in the training population with some measure of statistical confidence, for example a *Mest* having *ap* value of 0.05, less than 0.01, less than 0.005, less than 0.001, less than 0.0005, less than 0.0001, less than 0.00005, less than 0.00001, or less. Then, ratios of molecular markers can be formed using one molecular marker from the first set and a second molecular marker from the second set.

In another embodiment, a ratio for use in a selected combination is one in which the numerator represents the molecular marker data that demonstrates the molecular marker is upregulated in a first trait subgroup (as compared with a second trait subgroup) and the denominator represents the molecular marker data that demonstrates the molecular marker is upregulated in a second trait subgroup (as compared with a first trait subgroup). With such a ratio, a value greater than "1" indicates that the organism from which the molecular marker data was measured is a member of the first trait subgroup whereas a value less than "1" indicates that this organism is a member of the second trait subgroup. Thus, in some embodiments, step 214 comprises obtaining combinations of ratios of

molecular marker data. In some embodiments, step 214 comprises obtaining some multiple of molecular markers and forming a plurality of ratios of the molecular marker data so as to generate a plurality of combinations of molecular markers.

In step 214, a combination of molecular markers is selected. In some
5 embodiments, this combination of molecular markers consists of a single molecular marker. In some embodiments, this combination of molecular markers comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, as many as 30, as many as 40, as many as 50 or more molecular markers. In some embodiments, this combination of molecular markers consists of a combination of ratios of molecular markers wherein the
10 combination comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, as many as 30, as many as 40, as many as 50 or more molecular markers. For each candidate molecular marker added, the number of possible combinations grows exponentially. The limitation to the number of combinations selected for evaluation is dependent upon the capacity of the computer, network of computers or supercomputers utilized. In one
15 embodiment, all possible combinations of molecular markers resulting from steps 202-213 (or resulting from some subset of steps 202-213) are chosen, in another embodiment, all possible combinations of ratios of molecular markers resulting from steps 202-213 (or resulting from some subset of steps 202-213) are chosen. In another embodiment, one can subject all possible pairs of candidate molecular markers; all possible combinations of
20 three molecular markers, all possible combinations of four molecular markers; all possible combinations of five molecular markers, all possible combinations of six molecular markers, all possible combinations of seven molecular markers etc. In another embodiment, all possible combinations of two sets of ratios are chosen, in another embodiment, all possible combinations of three sets of ratios are chosen, in another
25 embodiment, all possible combinations of four sets of ratios are chosen, in another embodiment, all possible combinations of five sets of ratios are chosen. Each of the combinations of molecular markers is evaluated in subsequent processing steps.

Step 216.

In step 216, a classifier is computed using each combination of molecular markers
30 chosen in the last instance of step 214 and by applying the classifier to the molecular marker data measured for each molecular marker of this combination of molecular markers to a mathematical model, such as the mathematical models defined in Section 5.14 resulting in one or more classifiers for each combination. As described more thoroughly in Step 204, in some embodiments, one or more subjects of the training population are

identified as outliers and are removed prior to computing classifiers for each combination of molecular markers chosen.

In order to compute a classifier, in some embodiments, the mathematical model is a regression model, a neural network, a clustering model, principal component analysis, nearest neighbor classifier analysis, linear discriminant analysis, quadratic discriminant analysis, a support vector machine, a decision tree, a genetic algorithm, classifier optimization using bagging, classifier optimization using boosting, classifier optimization using the Random Subspace Method, Bayesian Networks (see F. V. Jensen. "Bayesian Networks and Decision Graphs". Springer. 2001, which is incorporated herein by reference in its entirety), a projection pursuit, weighted voting, a ratio or combination of ratios, or any combination of the above. Representative mathematical models that can be used in the present invention are described in Section 5.14. In the case where the mathematical model is a ratio or combination of ratios, steps 214 and 216 involve using the low throughput molecular marker data from training population 44 that was measured in step 206 to determine which molecular markers should be in the numerator of the ratios and which molecular markers should be in the denominator of the ratios. In some embodiments, the mathematical model used comprises a plurality of ratios of the molecular marker data. In such embodiments, the molecular marker data used in the numerators of the plurality of ratios can be the same or different than the molecular marker data used in the denominators of the plurality of ratios. In other words, a given molecular marker can be represented in the numerator of more than one ratio in the plurality of ratios or represented in the denominator of more than one ratio in the plurality of ratios.

Step 218.

In optional step 218 a determination is made as to whether all of the possible desired combinations of molecular markers to be tested have been considered. As discussed above in step 214, all or a portion of possible combinations may be tested. If not (218-No) process control returns to step 214 where another combination of molecular markers is selected and, at step 216, this new combination of molecular markers is evaluated using a mathematical model applied to the molecular marker data of the new combination. In some embodiments, the candidate molecular marker list comprises less than 25, less than 24, less than 23, less than 22, less than 21, or less than 20 molecular markers at step 214. In some embodiments, step 218 requires that a classifier be computed for all possible combinations of molecular markers. In other embodiments, step 218

requires that classifiers for only a portion of the possible combinations of molecular markers be considered.

In some embodiments, some aspects of steps 214-218 are performed by molecular marker classifier evaluation module 62. In fact, in some embodiments, several
5 different software programs, such as Microsoft (Redmond, Washington) Excel, are used in steps 214-218.

Step 220.

Once all the desired classifiers have been computed by loop 214-218, the classifiers are evaluated to determine which of the classifiers are most effective. In one embodiment
10 the resulting classifiers of loop 214-218 are scored. In some embodiments, scoring is done using the training population 44. In other embodiments, scoring is done using a "scoring population" wherein the scoring population includes at least some members not present in the training population. In one embodiment, the scoring population includes members of the training population in addition to one or more members not used in the training
15 population. In some embodiments, five percent or less, ten percent or less, twenty percent or less, thirty percent or less, fifty percent or less, or ninety percent or less of the members of the training population are common to the scoring population.

In some embodiments, the Percent Correct Predictions statistic is used to score each classifier. The "Percent Correct Predictions" statistic assumes that if the estimated p
20 is greater than or equal to 0.5, then the event is expected to occur and to not occur otherwise. By assigning these probabilities zeros and ones, a comparison can be made to the values of the samples in the training population to determine what percentage of the training population was sampled correctly.

In some embodiments, ROC analysis is performed and is used to score the
25 classifiers. In one embodiment, the area under the ROC curve is used to judge the quality of the classifier. As would be understood by those of skill in the relevant arts, area under the curve converts the two dimensional information contained in the ROC curve into one dimensional information. In other embodiments, information from the two dimensional aspect of the ROC curve is utilized directly. For example, the ROC curve also provides
30 information with respect to the sensitivity and specificity of the classifier. In some embodiments, classifiers are selected on the basis of either sensitivity or specificity. This can be an important scoring indicator. For example, a diagnostic classifier with high sensitivity (ie high true positive rate and low false negative rate may be important in situations where it is safer to misdiagnosis an individual as having disease rather than

misdiagnosing a disease bearing person as normal. Therefore in some embodiments, a cutoff can be set for either sensitivity or specificity and the classifier ranked or scored on the basis of the remaining variable. In some embodiments, ROC curves are generated for each model computed in an instance of step 216 using data obtained in step 206 for

5 members of either the training population or scoring population or both.

In some embodiments, the classifier resulting from the application of the mathematical itself results in a score as to the accuracy of the model. In this embodiment, the score is based on the accuracy of the model within the training population only.

In some embodiments, a classifier is a weighted logistic regression model

10 characterized by a multcategory logit model. For example, in some embodiments, a classifier discriminates between two different trait groups. In other embodiments, a classifier discriminates between more than two different trait groups. Logit models, including multcategory logit models are described in Agresti, *An Introduction to Categorical Data Analysis*, John Wiley & Sons, Inc., 1996, New York, Chapters 7 and 8,

15 which is hereby incorporated by reference. Table E illustrates the data that is used to form an ROC curve based on expression data applied to a mathematical model that uses the logit:

$$\ln[p/(1-p)] = \alpha + \beta_1 X_1 + \beta_2 X_2 + \epsilon$$

Table E: Values for the logit $\ln[p/(1-p)] = \alpha + \beta_1 X_1 + \beta_2 X_2 + \epsilon$ using hypothetical

20 values for training population 44

$\ln[p/(1-p)]$	Presence / Absence of a Trait
0.98	Y
0.97	Y
0.95	Y
0.93	Y
0.91	N
0.11	Y
0.07	N
0.03	N

Each row in Table E corresponds to a different specimen in the scoring population. The left column represents the results of the logit for the classifier being sampled. The

25 specimens in Table E are ranked by the logit score listed in the left hand column. The

right hand column details the presence or absence of the trait that is being considered by the regression equation. Table E can be used to compute a ROC curve using the same techniques disclosed in step 208 (in which each row in Table E is considered a threshold cutoff value in order to compute ROC curve datapoints). Then, the area under the ROC curve can be computed in order to assess the predictive quality of the classifier.

In step 220, each classifier is scored using any of the techniques disclosed here or that are known in the art. The classifiers can then be ranked based on their score. For example, they can be ranked based on the percent correct predictions, area under the ROC curve, sensitivity or specificity or some weighted or unweighted combination of the two scoring techniques. In some embodiments, step 220 is performed by molecular marker evaluation module 64.

Step 222.

Step 222 is optional. Optional step 222 provides additional filtering to eliminate some of the candidate classifiers computed in loop 214-218. In one such filter, limited to the case in which the classifiers computed in steps 214-218 are based on application of data to regression based mathematical models, classifiers that have at least one coefficient that is large are eliminated. Such classifiers have the potential to magnify small errors in the data. In some embodiments, determination as to whether or not a coefficient is large can require multiple computation steps, in instances where a coefficient uniquely represents a molecular marker, the maximum value (MAX) for the data measured for the molecular marker in a trait subgroup associated with the classifier is identified. For example, consider the case in which a given coefficient uniquely represents the expression of gene A in blood. Further suppose that low throughput data for gene A from 10 individuals of a particular trait subgroup was measured in step 206. The value MAX would be the largest expression value observed for gene A in the ten individuals from the subject trait subgroup. For example, if individual #7 in the set of ten individuals exhibited the highest expression level for gene A as determined by the methods of step 206, then the expression value measured for gene A in individual #7 will represent MAX. Next, the minimum value (MIN) for the data measured for the molecular marker in the subject trait subgroup is identified. In the example presented above, MIN is the expression level of gene A in the subject having the lowest expression level for gene A in the set of ten subjects as determined by the low throughput measurement methods of step 206. Next, the coefficient derived in the regression for the unique molecular marker (e.g., the coefficient for gene A) is multiplied by the difference between (MAX) and (MIN) in order to obtain

the test value (TEST). In other words, for each coefficient i in a classifier, the following equation is computed:

$$\text{TEST}_i = \text{coefficient}_i * [\text{MAX} - \text{MIN}].$$

As an example, consider the case in which a classifier is used to determine whether
 5 or not a subject has a particular cancer. In this case, one of the trait subgroups in training
 population 44 will represent patients that have this cancer. To evaluate a coefficient of a
 classifier in this case, the low value and high value for the measured data of the molecular
 marker i in the trait subgroup is obtained and the difference between these two values is
 multiplied against the coefficient value in order to obtain the value of TEST,. In some
 10 embodiments, a coefficient i is considered large and the classifier that includes the
 coefficient is discarded when the value is greater than 5, greater than 10, greater than 100
 or greater than 1000.

In typical embodiments, a classifier will determine whether a subject falls into one
 of at least two different trait subgroups. In other words, the classifier will discriminate
 15 between at least two different trait subgroups. A test has been presented above for
 determining whether a coefficient in a regression derived classifier is too large. This test
 used one of the trait subgroups that the classifier discriminates. In some embodiments, the
 test is repeated for each of the trait subgroups that the classifier can discriminate. For
 example, in the case of a classifier that can discriminate between the cancerous trait
 20 subgroup and the non-cancerous trait subgroup, the test is independently run using data
 from each trait subgroup. That is, the test is first run using only data from the cancerous
 trait subgroup and then the test is run a second time using only data from the non-
 cancerous trait subgroup. If a coefficient is too large in any such independent test, the
 classifier is eliminated from further consideration. In some embodiments, the test is run
 25 against only one of the possible trait subgroups that the subject classifier can discriminate.

In another embodiment, some classifiers are eliminated on the basis of the score.
 For example, where the scoring system used is receiver operating characteristic (ROC)
 curve score determined by an area under the ROC curve, in some embodiments, those
 classifiers with scores of less than 0.95, 0.9, 0.85, 0.8, 0.7, 0.65, 0.6, 0.55 0.5 or 0.45 or
 30 less can be eliminated. In other embodiments, where specificity is important to the use of
 the classifier, a sensitivity threshold can be set and classifiers ranked on the basis of the
 specificity. for example with a cutoff for specificity of less than 0.95, 0.9, 0.85, 0.8, 0.7,
 0.65, 0.6, 0.55 0.5 or 0.45 or less can be eliminated. Similarly, the specificity threshold
 can be set and classifiers ranked on the basis of sensitivity for example with a cutoff for

sensitivity of less than 0.95, 0.9, 0.85, 0.8, 0.7, 0.65, 0.6, 0.55, 0.5 or 0.45 or less can be eliminated. Thus in some embodiments, only the top 10 ranking classifiers, the top 20 ranking classifiers, or the top 100 ranking classifiers are selected and the remaining classifiers eliminated.

5 *Step 224.*

After classifiers have been scored and ranked and some classifiers optionally eliminated; one or more classifiers can be combined to create a classifier group. For instance, in some embodiments, the top 10 ranking classifiers, the top 20 ranking classifiers, or the top 100 ranking classifiers are selected. In some embodiments, any of
10 the top 1 to top 500 ranking classifiers is selected, in instances where more than one classifier is selected, in one embodiment, each classifier contributes one vote to the diagnosis of the test subject such that diagnosis of the test subject is determined as a result of a combination of classifiers. In other embodiments, multiple classifiers can be used and different weighting schema applied to each classifier. For example, weighting schema can
15 include weighting on the basis of factors such as the original score the classifier, the logs odd ratio ("logit"), the size of the coefficients for each classifier, some combination thereof and the like.

Step 226.

Step 226 is optional. Optional step 226 is useful if training population 44 is
20 comprised of more than two trait subgroups. In cases where there are more than two trait subgroups, multiple binary classifiers (or groups of said classifiers) can be developed wherein each binary classifier (or group of said classifiers) is directed towards differentiating as between two traits. In one embodiment, each round of the funnel (e.g. steps 202, 204, 206, 214, 216, 220 and 224) produces a set of binary classifiers. In another
25 embodiment, multiple lists of binary candidate molecular markers are developed by performing step 202, and then binary classifiers (or groups of binary classifiers) are developed by proceeding with multiple rounds of the remainder of the funnel (e.g. steps 204, 206, 214, 216, 220 and step 224). Because each classifier represents only a binary test e.g. the absence or presence of a single trait, in step 226 a determination is made as to
30 whether all classifiers have been developed for training population 44. If not (226-No), process control returns to step 210 and work is initiated to develop a classifier for a different trait represented by training population 44. Therefore, steps 210-224 can optionally be repeated until one or more classifiers or groups of classifiers have been selected for each of the trait subgroups represented by training population 44.

In some embodiments, each classifier or group of classifiers developed in accordance with embodiments of the present invention is stored in classifier database 70. Fig. 5 illustrates an exemplary classifier database 70 in accordance with one embodiment of the present invention. Database 70 includes an entry 400 for each classifier 400. Each classifier 400 is optionally given a classifier name 402. Each classifier 400 is part of classifier. For example, a given classifier can consist of only a single classifier. In other embodiments, a given classifier can consist of one or a plurality of classifiers. Therefore, each classifier 402 includes an indicator 403 to indicate which classifier the classifier is in. Further, each classifier 400 has an optional indicator 404 to indicate the trait that the classifier can discriminate. In some embodiments, optional indicator indicates the trait subgroups that the classifier can discriminate. In some embodiments such information can be inferred from the classifier identifier field 403 since each classifier represents the absence or presence of a particular trait (e.g., absence or presence of cancer). In addition to this header information, each classifier includes the identity 406 of one or more molecular markers and the respective coefficients 408 for each of the molecular markers.

Once classifiers or classifier groups have been developed, the classifiers can be used to diagnose a patient that has presented as possibly having a disease that can be differentiated by the classifiers. Figure 3 is a flowchart of a method of applying the classifiers to a patient.

20 *Step 328.*

Step 328 can be performed after the previously described steps, or can be used in conjunction with a classifier or classifier groups derived using the methods disclosed herein. As such, steps 328-334 represent a completely independent method of the present invention and can be performed at any time once suitable classifiers have been developed using, for example, steps 301 through 326. Step 328 is used in conjunction with step 330 to diagnose a trait of interest of an individual not represented in either the training population or the scoring population (a "test individual"). Each classifier or classifier group identified previously can be used to determine whether a test individual has a trait of interest. In order to perform such tests, molecular marker data for each molecular marker of the classifier or classifier group of interest is required. To obtain such data, a sample of blood from the subject is obtained using any of the techniques described in Section 5.2. The sample is used to measure molecular marker data for each molecular marker in the sample using any of the techniques described in Sections 5.3 or 5.4. Thus in step 328, once classifiers have been identified, molecular marker data for use with the classifier or

classifier groups can be obtained using either high throughput or low throughput techniques.

Advantageously, the molecular marker data obtained in step 328 can be stored in patient database 68 for later use. In fact, in some embodiments, rather than obtaining
5 molecular marker data from a patient sample in step 328, the data is obtained from a subject in patient database 68. In such embodiments, the molecular marker data was previously loaded into patient database 68.

Fig. 6 illustrates a patient database 68 in accordance with one embodiment of the present invention. There is a record 500 for each patient (subject) tracked by patient
10 database 68. Each patient record 500 optionally includes a patient identifier 502 to uniquely identify the patient. In some embodiments such unique identifiers can be inferred from the patient record value 500. Each patient record 500 includes a molecular profile 504 comprising molecular marker data collected for a plurality of molecular marker products from a sample defined in Section 5.2 using any one of the techniques described in
15 Sections 5.3 and 5.4. In typical embodiments, a molecular profile 504 includes a plurality of molecular marker identities 506 and the corresponding measured molecular marker data values 508 for such molecular markers. In addition to the molecular profile, each patient record 502 can include one or more traits 510. Such trait characterizations can be assigned by observation of the subject and/or by testing the patient's molecular profile using the
20 classifiers constructed in accordance with the methods of the present invention. Section 5.10, below, provides more details on exemplary patient databases 68.

Step 330.

In step 330 the classifier created using some or more of the previous steps is used to diagnose a test individual. In some embodiments diagnosis can be performed using a
25 classifier group from step 224. For example, in one embodiment, where there are numerous classifiers after step 222 that provide satisfactory scores (given the purpose for use), a test subject can be diagnosed by using the results of all or some of these classifiers in the form of a classifier group as described in step 224. In one embodiment, the term diagnosis means the results of a single classifier or group of classifiers resulting from the
30 application of the funneling method described in steps 202-224. For example, the resulting classifier or group of classifiers will enable the ability to determine whether a test individual belongs to one of two possible trait subgroups. In another embodiment, by the term diagnosis is meant the results of multiple classifiers or multiple groups of classifiers (ie classifiers resulting from the application of more than one round of the funneling

method described in steps 202 —224). For example, the resulting classifiers or groups of classifiers used in series can allow a diagnosis as to whether an individual belongs to one of three or more possible trait subgroups (e.g. results of first classifier distinguish as to whether person has schizophrenia or does not have schizophrenia - If not schizophrenia
 5 apply a second classifier or group of classifiers to determine whether individual has bipolar disorder or does not have bipolar disorder etc.) The use of the classifiers to diagnose depends upon the trait subgroups used to develop the classifier. For example, if the classifier was developed to differentiate as between two trait subgroups, the classifier can be used to diagnose a test subject as being either of the first trait subgroup or the second
 10 trait subgroup. To diagnose a test subject, preferably a quantitative technique such as quantitative RT-PCR is utilized to obtain molecular marker data measured in step 328 is used.

To illustrate the use of a classifier to diagnose, consider the case in which the classifier comprises the classifier group:

$$15 \quad \ln[p/(1-p)] = 0.34 + 0.24JT_1 + 0.74X_3 + 0.03 ,$$

$$\ln[p/(1-p)] = 0.54 - OAST_2 + 83X_3 + 0.01 .$$

That is, the exemplary classifier group consisting of two classifiers. To poll the classifier, the data (e.g. abundance level, activity level, etc.) for molecular markers X_1 , X_2 and X_3 is measured using any of the techniques described in Section 5.3 or 5.4. Then,
 20 these data measurement values are placed into the classifier equations and the equations are processed and the output is used to predict outcome. In one embodiment, classifier equation values that approach a value of one indicate that the sample has the trait associated with the classifier whereas classifier equation values that approach zero indicate that the sample does not have the trait associated with the classifier. In other
 25 embodiments, the equations are regressed so that the opposite relationship hold (e.g., equation values approach one indicate absence of an associated trait). In one embodiment, each equation is assigned a "+1" vote if the equation approaches one or a "-1" vote if the equation approaches zero. Equation votes are summed. If the net summation is positive, then the subject is deemed to have the trait associated with the classifier. If the net
 30 summation is negative, then the subject is deemed not to have the trait associated with the classifier. In some embodiments, step 330 is performed by model polling and reporting module 66.

Step 332.

One of the advantages of the present invention is that a single sample collected in accordance with Section 5.2 can be used to test the patient for one or more of a plurality of molecular markers which may be useful for one or more traits. Accordingly, in step 332, a determination is made as to whether the patient has been tested for each pair of traits for which a determination is required. If additional determinations are required (332-No), process control is returned to step 330 and the measured molecular marker data from the patient is used to help determine the likelihood as to whether a subject has other traits represented by a trait subgroup in training population 44.

Step 334.

When the patient sample has been used to determine if the subject has any of a plurality of different traits as determined by one or more classifiers or classifier groups, a report is generated. In some embodiments, this report includes the results of each classification test. In other words, the report provides an indication as to whether it is likely the tested subject has any one of a plurality of different traits. In some embodiments, step 334 is performed by classifier polling and reporting module 66. Section 5.5 provides a summary of some of the applications for classifiers constructed using the methods of the present invention.

5.2 SOURCE OF MOLECULAR MARKER DATA

The present invention provides methods for identifying molecular markers by obtaining molecular marker data which represents the products of molecular markers found in a blood sample. Molecular markers are thus identified that correlate with, are associated with, or indicate a trait. The present invention also provides methods for detecting, diagnosing, monitoring, prognosing or predicting a trait or reoccurrence of a trait based upon data corresponding to the expression of molecular markers in a blood sample. As used herein, the terms "subject" and "patient" and "individual" are used interchangeably to refer to an animal (e.g., a mammal, a fish, an amphibian, a reptile, a bird, and an insect). In a specific embodiment, a subject is a mammal (e.g., a non-human mammal and a human). In another embodiment, a subject is a pet (e.g., a dog, a cat, a guinea pig, a monkey and a bird), a farm animal (e.g., a horse, a cow, a pig, a goat and a chicken) or a laboratory animal (e.g., a mouse and rat). In another embodiment, the subject is a primate (e.g., a chimpanzee and a human). In a preferred embodiment, the subject is a human.

5.2.1 SOURCE OF A BLOOD SAMPLE

A blood sample obtained from any subject may be used in accordance with the methods of the invention. Examples of subjects from which a blood sample can be obtained and utilized in accordance with the methods of the invention include, but are not limited to, asymptomatic subjects, subjects manifesting or exhibiting 1, 2, 3, 4 or more traits or symptoms of a trait, subjects clinically diagnosed as having a trait, subjects predisposed to a trait (*e.g.*, subjects with a family history of a trait, subjects with a genetic predisposition to a trait, and subjects that lead a lifestyle that predisposes them to a trait or increases the likelihood of contracting a trait), subjects suspected of having a trait, subjects undergoing therapy for a trait, subjects non-responsive to a therapy, subjects responsive to a therapy, subjects with more than one trait (*e.g.*, subjects with 2, 3, 4, 5 or more traits), subjects not undergoing therapy for a trait, subjects determined by a medical practitioner (*e.g.*, a physician) to be healthy or disease-free, subjects that are in remission, subjects cured of trait, and subjects that have not been diagnosed with a condition. In specific embodiment, the condition is a disease. In another specific embodiment, a condition is any state that is codified in the *International Classification of Diseases*, 9th Revision, Department of Health and Human Services (ICD-9 codes) and/or SNOMED Clinical Terms (SNOMED CT®) which is hereby incorporated by reference, or equivalent treatise.

Non-limiting examples of disease include, but are not limited to, blood disorder, blood lipid disease, autoimmune disease, arthritis (*e.g.*, osteoarthritis, rheumatoid arthritis, juvenile rheumatoid arthritis and the like), bone or joint disorder, lupus, an allergy, a cardiovascular disorder (*e.g.*, heart failure, congenital heart disease, rheumatic fever, valvular heart disease, cor pulmonale, cardiomyopathy, myocarditis, pericardial disease, vascular diseases such as atherosclerosis, acute myocardial infarction, ischemic heart disease and the like), obesity, respiratory disease (*e.g.*, asthma, pneumonitis, pneumonia, pulmonary infections, lung disease, bronchiectasis, tuberculosis, cystic fibrosis, interstitial lung disease, chronic bronchitis emphysema, pulmonary hypertension, pulmonary thromboembolism, acute respiratory distress syndrome and the like), hyperlipidemias, endocrine disorder, immune disorder, infectious disease, muscle wasting and whole body wasting disorder, neurological disorder (*e.g.*, migraines, seizures, epilepsy, cerebrovascular disease, Parkinson's, ataxic disorders, motor neuron diseases, cranial nerve disorders, spinal cord disorders, meningitis and the like), neurodegenerative disease (*e.g.*, alzheimers, dementia and the like), neuropsychiatric disease (*e.g.*, schizophrenia, anxiety and the like), mood disorders (*e.g.*, bipolar disorder; manic depression and the like), skin disorder,

kidney disease, scleroderma, stroke, hereditary hemorrhage telangiectasia, diabetes, disorders associated with diabetes (e.g., PVD), hypertension, Gaucher's disease, cystic fibrosis, sickle cell anemia, liver disease, stomach disease, pancreatic disease, eye disease, ear disease, nose disease, throat disease, diseases affecting the reproductive organs, gastrointestinal diseases (including diseases of the colon, diseases of the spleen, appendix, gall bladder, and others) and the like. For further discussion of human diseases, see Mendelian Inheritance in Man: A Catalog of Human Genes and Genetic Disorders by Victor A. McKusick (12th Edition, 3 volume set, June 1998, Johns Hopkins University Press, ISBN: 0801857422) and Harrison's Principles of Internal Medicine by Braunwald, Fauci, Kasper, Hauser, Longo, & Jameson (15th Edition 2001), the entirety of each of which is incorporated herein. Additional examples of disease are disclosed in Section 5.8, below.

In one embodiment of the invention, the disease is an immune disorder, such as those associated with overexpression of a gene or expression of a mutant gene (e.g., autoimmune diseases, such as diabetes mellitus, arthritis (including rheumatoid arthritis, juvenile rheumatoid arthritis, osteoarthritis, and psoriatic arthritis), multiple sclerosis, encephalomyelitis, myasthenia gravis, systemic lupus erythematosus, automimmune thyroiditis, dermatitis (including atopic dermatitis and eczematous dermatitis), psoriasis, Sjogren's Syndrome, Crohn's disease, aphthous ulcer, iritis, conjunctivitis, keratoconjunctivitis, ulcerative colitis, asthma, allergic asthma, cutaneous lupus erythematosus, scleroderma, vaginitis, proctitis, drug eruptions, leprosy reversal reactions, erythema nodosum leprosum, autoimmune uveitis, allergic encephalomyelitis, acute necrotizing hemorrhagic encephalopathy, idiopathic bilateral progressive sensorineural hearing, loss, aplastic anemia, pure red cell anemia, idiopathic thrombocytopenia, polychondritis, Wegener's granulomatosis, chronic active hepatitis, Stevens-Johnson syndrome, idiopathic sprue, lichen planus, Graves' disease, sarcoidosis, primary biliary cirrhosis, uveitis posterior, and interstitial lung fibrosis), graft-versus-host disease, cases of transplantation, and allergy.

In another embodiment, a disease of the invention is a cellular proliferative and/or differentiative disorder that includes, but is not limited to, cancer, e.g., carcinoma, sarcoma or other metastatic disorders and the like. As used herein, the term "cancer" refers to cells having the capacity for autonomous growth, i.e., an abnormal state of condition characterized by rapidly proliferating cell growth. "Cancer" is meant to include all types of cancerous growths, pre-cancerous growths or lesions, oncogenic processes, metastatic

tissues or malignantly transformed cells, tissues, or organs, irrespective of histopathologic type or stage of invasiveness. Examples of cancers include, but are not limited to, solid tumors, tissue specific tumors, benign cancer, metastatic cancers, early stage cancer, late stage cancer and leukemias, including: apudoma, choristoma, branchioma, malignant

5 carcinoid syndrome, carcinoid heart disease, carcinoma (e.g., Walker, basal cell, basosquamous, Brown-Pearce, ductal, Ehrlich tumour, in situ, Krebs 2, Merkel cell, mucinous, non-small cell lung, oat cell, papillary, scirrhous, bronchiolar, bronchogenic, squamous cell, and transitional cell), histiocytic disorders, leukaemia (e.g., B cell, mixed

10 cell, null cell, T cell, T-cell chronic, HTLV-II-associated, lymphocytic acute, lymphocytic chronic, mast cell, and myeloid), histiocytosis malignant, Hodgkin disease, immunoproliferative small, non-Hodgkin lymphoma, plasmacytoma, reticuloendotheliosis, melanoma, chondroblastoma, chondroma, chondrosarcoma, fibroma, fibrosarcoma, giant cell tumors, histiocytoma, lipoma, liposarcoma, mesothelioma, myxoma, myxosarcoma, osteoma, osteosarcoma, Ewing sarcoma, synovioma, adenofibroma, adenolymphoma,

15 carcinosarcoma, chordoma, craniopharyngioma, dysgerminoma, hamartoma, mesenchymoma, mesonephroma, myosarcoma, ameloblastoma, cementoma, odontoma, teratoma, thymoma, trophoblastic tumour, adeno-carcinoma, adenoma, cholangioma, cholesteatoma, cylindroma, cystadenocarcinoma, cystadenoma, granulosa cell tumour, gynandroblastoma, hepatoma, hidradenoma, islet cell tumour, Leydig cell tumour,

20 papilloma, Sertoli cell tumour, theca cell tumour, leiomyoma, leiomyosarcoma, myoblastoma, myoma, myosarcoma, rhabdomyoma, rhabdomyosarcoma, ependymoma, ganglioneuroma, glioma, medulloblastoma, meningioma, neurilemmoma, neuroblastoma, neuroepithelioma, neurofibroma, neuroma, paraganglioma, paraganglioma nonchromaffin, angiokeratoma, angiolymphoid hyperplasia with eosinophilia, angioma sclerosing,

25 angiomatosis, glomangioma, hemangioendothelioma, hemangioma, hemangiopericytoma, hemangiosarcoma, lymphangioma, lymphangiomyoma, lymphangiosarcoma, pinealoma, carcinosarcoma, chondrosarcoma, cystosarcoma, phyllodes, fibrosarcoma, hemangiosarcoma, leiomyosarcoma, leukosarcoma, liposarcoma, lymphangiosarcoma, myosarcoma, myxosarcoma, ovarian carcinoma, rhabdomyosarcoma, sarcoma (e.g.,

30 Ewing, experimental, Kaposi, and mast cell), neoplasms (e.g., bone, breast, digestive system, colorectal, liver, pancreatic, pituitary, testicular, orbital, head and neck, central nervous system, acoustic, pelvic respiratory tract, and urogenital), neurofibromatosis, and cervical dysplasia, and other conditions in which cells have become immortalized or transformed.

5.2.2 METHODS FOR COLLECTING BLOOD

In one aspect, a sample of blood is obtained from a subject according to methods well known in the art. The present invention can use whole blood, but can also use blood in which the serum or plasma has been removed and the RNA or mRNA isolated from the remaining sample in accordance with methods known in the art (for example, using
5 preferably gentle centrifugation at 300-800xg for five to ten minutes).

In some embodiments a drop of blood is collected from a simple pin prick made in the skin of a subject. In such embodiments, this drop of blood collected from a pin prick is all that is needed. A drop of blood can include volumes of anywhere from 10uL through to
10 100uL. Blood may be drawn from a subject from any part of the body (*e.g.*, a finger, a hand, a wrist, an arm, a leg, a foot, an ankle, a stomach, and a neck) using techniques known to one of skill in the art, in particular methods of phlebotomy known in the art. In a specific embodiment, venous blood is obtained from a subject and utilized in accordance with the methods of the invention. In another embodiment, arterial blood is obtained and
15 utilized in accordance with the methods of the invention. The composition of venous blood varies according to the metabolic needs of the area of the body it is servicing. In contrast, the composition of arterial blood is consistent throughout the body. For routine blood tests, venous blood is generally used.

Venous blood can be obtained from any source including the basilic vein, cephalic
20 vein, or median vein. Arterial blood can be obtained from the radial artery, brachial artery or femoral artery. A vacuum tube, a syringe or a butterfly may be used to draw the blood. Typically, the puncture site is cleaned, a tourniquet is applied approximately 3-4 inches above the puncture site, a needle is inserted at about a 15-45 degree angle, and if using a vacuum tube, the tube is pushed into the needle holder as soon as the needle penetrates the
25 wall of the vein. When finished collecting the blood, the needle is removed and pressure is maintained on the puncture site. Usually, heparin or another type of anticoagulant is in the tube or vial that the blood is collected in so that the blood does not clot. When collecting arterial blood, anesthetics can be administered prior to collection.

The amount of blood collected will vary depending upon the site of collection, the
30 amount required for a method of the invention, and the comfort of the subject. However, an advantage of one embodiment of the present invention is that the amount of blood required to implement the methods of the present invention can be so small that more invasive procedures are not required to obtain the sample. For example, in some embodiments, all that is required is a drop of blood. This drop of blood can be obtained,

for example, from a simple pinprick. In some embodiments, any amount of blood is collected that is sufficient to measure molecular marker data. As such, in some embodiments, the amount of blood that is collected is 1 μ l or less, 0.5 μ l or less, 0.1 μ l or less, or 0.01 μ l or less. However, the present invention is not limited to such

5 embodiments. In some embodiments more blood is available and in some embodiments, more blood can be used to effect the methods of the present invention. As such, a broad range of blood volumes is contemplated and can be used to obtain the molecular marker data measurement data used in the present invention. In various specific embodiments, 0.001 ml, 0.005 ml, 0.01 ml, 0.05 ml, 0.1 ml, 0.15 ml, 0.2 ml, 0.25 ml, 0.5 ml, 0.75 ml, 1

10 ml, 1.5 ml, 2 ml, 3 ml, 4 ml, 5 ml, 10 ml, 15 ml or more of blood is collected from a subject. In other specific embodiments, 0.001 ml to 15ml, 0.01 ml to 10 ml, 0.1 ml to 10 ml, 0.1 ml to 5 ml, 1 to 5 ml of blood is collected from a subject.

In some embodiments of the present invention, blood is stored within a K3/EDTA tube. In another embodiment, one can utilize tubes for storing blood which contain

15 stabilizing agents such as disclosed in United States patent No. 6,617,170. In another embodiment, the PAXgene™ blood RNA system provided by PreAnalytiX, a Qiagen/BD company, can be used to collect blood. In yet another embodiment, the Tempus™ blood RNA collection tubes, offered by Applied Biosystems, can be used. Tempus™ collection tubes provide a closed evacuated plastic tube containing RNA stabilizing reagent for

20 whole blood collection.

The collected blood is optionally but preferably stored at refrigerated temperatures, such as 4 °C, prior to molecular marker data measurement. In some embodiments, a portion of the blood sample is used for molecular measurement at a first instance of time whereas one or more remaining portions of the blood sample is stored for a period of time

25 for later use. This period of time can be an hour or more, a day or more, a week or more, a month or more, a year or more, or indefinitely. For long term storage, storage methods well known in the art, such as storage at cryo temperatures (*e.g.* below -60 °C) can be used. In some embodiments, in addition to storage of the blood (or instead of storage of the blood), isolated molecular markers (*e.g.*, nucleic acid, protein, carbohydrates, lipids, metabolites, *etc.*) are stored for a period of time for later use. Storage of such molecular

30 markers can be for an hour or more, a day or more, a week or more, a month or more, a year or more, or indefinitely.

5.2.3 METHOD OF ISOLATING BLOOD CELLS

In some embodiments of the present invention, whole blood is used directly to isolate and analyze the products of one or more molecular markers so as to obtain molecular marker data. In other embodiments of the invention fractionated blood can be used. By fractionated blood is meant blood in which the blood cells are separated prior to isolation of the molecular markers using techniques known in the art. For example, fractionated blood includes blood wherein the blood cells are fractionated using Ficoll-Hypaque (Pharmacia) gradient centrifugation. Such centrifugation separates erythrocytes (red blood cells) from various types of nucleated cells and from plasma. As such, in some embodiments of the present invention, a blood sample of the invention is fractionated blood. In one embodiment, peripheral blood leukocytes are utilized ("PBLs"). PBLs are separated from the remainder of the blood using a Ficoll® gradient.

By way of example but not limitation, macrophages can be obtained as follows. Mononuclear cells are isolated from peripheral blood of a subject, by syringe removal of blood followed by Ficoll-Hypaque gradient centrifugation. Tissue culture dishes are pre-coated with the subject's own serum or with AB+ human serum and incubated at 37°C for one hour. Non-adherent cells are removed by pipetting. Cold (4°C) Imm EDTA in phosphate-buffered saline is added to the adherent cells left in the dish and the dishes are left at room temperature for fifteen minutes. The cells are harvested, washed with RPMI buffer and suspended in RPMI buffer. Increased numbers of macrophages can be obtained by incubating at 37°C with macrophage-colony stimulating factor (M-CSF). Antibodies against macrophage specific surface markers, such as Mac-1_s can be labeled by conjugation of an affinity compound to such molecules to facilitate detection and separation of macrophages. Affinity compounds that can be used include but are not limited to biotin, photobiotin, fluorescein isothiocyanate (FITC), or phycoerythrin (PE), or other compounds known in the art. Cells retaining labeled antibodies are then separated from cells that do not bind such antibodies by techniques known in the art such as, but not limited to, various cell sorting methods, affinity chromatography, and panning.

Blood cells can be fractionated using a fluorescence activated cell sorter (FACS). Fluorescence activated cell sorting (FACS) is a known method for separating particles, including cells, based on the fluorescent properties of the particles. See, for example, Kamarch, 1987, Methods Enzymol 151:150-165. Laser excitation of fluorescent moieties in the individual particles results in a small electrical charge allowing electromagnetic separation of positive and negative particles from a mixture. An antibody

or ligand used to detect a blood cell antigenic determinant present on the cell surface of particular blood cells is labeled with a fluorochrome, such as FITC or phycoerythrin. The cells are incubated with the fluorescently labeled antibody or ligand for a time period sufficient to allow the labeled antibody or ligand to bind to cells. The cells are processed
5 through the cell sorter, allowing separation of the cells of interest from other cells. FACS sorted particles can be directly deposited into individual wells of microtiter plates to facilitate separation.

Magnetic beads can be also used to separate blood cells in some embodiments of the present invention. For example, blood cells can be sorted using a magnetic activated
10 cell sorting (MACS) technique, a method for separating particles based on their ability to bind magnetic beads (0.5 100 Dm diameter). A variety of useful modifications can be performed on the magnetic microspheres, including covalent addition of an antibody which specifically recognizes a cell solid phase surface molecule or hapten. A magnetic field is then applied, to physically manipulate the selected beads. In a specific
15 embodiment, antibodies to a blood cell surface marker are coupled to magnetic beads. The beads are then mixed with the blood cell culture to allow binding. Cells are then passed through a magnetic field to separate out cells having the blood cell surface markers of interest. These cells can then be isolated.

In some embodiments, the surface of a culture dish may be coated with antibodies,
20 and used to separate blood cells by a method called panning. Separate dishes can be coated with antibody specific to particular blood cells. Cells can be added first to a dish coated with blood cell specific antibodies of interest. After thorough rinsing, the cells left bound to the dish will be cells that express the blood cell markers of interest. Examples of cell surface antigenic determinants or markers include, but are not limited to, CD2 for T
25 lymphocytes and natural killer cells, CD3 for T lymphocytes, CD1 Ia for leukocytes, CD28 for T lymphocytes, CD 19 for B lymphocytes, CD20 for B lymphocytes, CD21 for B lymphocytes, CD22 for B lymphocytes, CD23 for B lymphocytes, CD29 for leukocytes, CD 14 for monocytes, CD41 for platelets, CD61 for platelets, CD66 for granulocytes, CD67 for granulocytes and CD68 for monocytes and macrophages.

30 Whole blood can also be fractioned into cells types such as leukocytes, platelets, erythrocytes, etc. Leukocytes can be further separated into granulocytes and agranulocytes using standard techniques. Granulocytes can be separated into cell types such as neutrophils, eosinophils, and basophils using standard techniques. Agranulocytes can be separated into lymphocytes (e.g., T lymphocytes and B lymphocytes) and monocytes using

standard techniques. T lymphocytes can be separated from B lymphocytes and helper T cells separated from cytotoxic T cells using standard techniques. Separated blood cells (e.g., leukocytes) can be frozen by standard techniques prior to use in the present methods.

5.2.4 BLOOD SAMPLES USED IN METHODS OF THE INVENTION

5 In accordance with the methods of the invention, the term "blood sample" can include any of the samples discussed in section 5.2.3. In some embodiments, this includes whole blood, fractionated blood, a sample of subsets of fractionated blood, and a sample of specific types of blood cells. In a specific embodiment, the whole blood sample can have the plasma or serum removed by centrifugation, using preferably gentle
10 centrifugation at 300-800xg for five to ten minutes.

 In another embodiment, a blood sample of the invention is a sample of peripheral blood leukocytes (PBLs). In another embodiment, a blood sample of the invention is a sample of granulocytes. In another embodiment, a blood sample of the invention is a sample of neutrophils, eosinophils, basophils or any combination thereof. In another
15 embodiment, a blood sample of the invention is a sample of agranulocytes. In another embodiment, a blood sample of the invention is a sample of lymphocytes, monocytes or a combination thereof. In yet another embodiment, a blood sample of the invention is a sample of T lymphocytes, B lymphocytes or a combination thereof. See, e.g., Section 5.4.3 supra for methods of isolating blood cells.

20 A blood sample that is useful according to the invention is in an amount that is sufficient for the detection of one or more molecular markers according to the invention. In a specific embodiment, a blood sample useful according to the invention is in an amount ranging from 1 µl to 100 ml, preferably 10 µl to 50 ml, more preferably 10 µl to 25 ml and most preferably 10 µl to 1 ml.

25 In one embodiment whole blood, or serum free whole blood is taken and the red blood cells are lysed with lysing buffer. In a specific embodiment, the Lysis Buffer (IL) consists of 0.6g EDTA 1.0g KHCO₂, and 8.2g NH₄Cl adjusted to pH 7.4 (using NaOH). Once mixed with lysing buffer, the sample is centrifuged and the cell pellet retained and RNA or mRNA extracted in accordance with methods known in the art
30 ("lysed whole blood") (see, for example, Sambrook, Fritsch & Maniatis, "Molecular Cloning: A Laboratory Manual (1982); "DNA Cloning: A Practical Approach," Volumes I and II (D.N. Glover ed. 1985). The use of whole blood or lysed whole blood is preferred since it avoids the costly and time-consuming need to separate out the cell types within the blood (Liew et al. U.S. Patent Application No. US 2004/0014059). In another

embodiment, whole blood is stored and stabilized using PAXgene® tubes and RNA can be isolated using the PAXgene® RNA Isolation system. In yet another embodiment, RNA is isolated from whole blood which has been isolated using PAXgene® and additional globin reduction protocols followed.

5.3 METHODS FOR MEASURING MOLECULAR MARKER DATA

The techniques described in this section are particularly useful for obtaining molecular marker data for step 202 wherein the data is reflective of the products of the molecular marker -e.g. measurement of the abundance of RNA or protein products in blood corresponding to all of the molecular markers of the genome or "a portion thereof.

- 10 In particular, the techniques useful for step 202 are those techniques which allow the ability to comprehensively screen for candidate molecular markers quickly and effectively. These techniques preferably provide molecular marker data for a large number of molecular markers concurrently, thereby allowing greater ability to screen molecular markers corresponding to the entire genome, or a portion thereof in a short period of time.
- 15 In addition to the techniques described in this Section 5.3, any technique known to one of skill in the art to measure the abundance of RNA or protein corresponding to the entire genome or "a portion thereof" can be used to measure such data. In one embodiment, "a portion thereof" is data corresponding to the amount of RNA or protein expressed from more than 1,000, more than 2,000, more than 5,000, more than 10,000, more than 20,000, 20 more than 30,000 molecular markers. In another embodiment, the "a portion thereof" refers to at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% of the genome. See, e.g., Sambrook, Fritsch & Maniatis, 1982, *Molecular Cloning: A Laboratory Manual*; *DNA Cloning: A Practical Approach*, volumes I and II (D.N. Glover ed. 1985); *Oligonucleotide* 25 *Synthesis* Gait ed., 1984; *Nucleic Acid Hybridization*, Hames & Higgins eds., 1985; *Transcription and Translation*, Hames & Higgins eds., 1984, *Animal Cell Culture*, Freshney ed., 1986, *Immobilized Cells And Enzymes*, IRL Press, 1986, Perbal, 1984, *A Practical Guide To Molecular Cloning*, each of which is hereby incorporated by reference in its entirety. In one embodiment more than technique can be used to measure data for 30 each molecular marker to perform step 202.

5.3.1 RNA MEASUREMENT TECHNIQUES

Any technique known to one of skill in the art may be used to measure the level of expression of a molecular marker by measuring the amount of the product of the molecular

marker. In one embodiment the RNA in blood corresponding to the molecular marker is measured. By "corresponding to a molecular marker" is meant RNA transcribed from a molecular marker (or proteins translated from RNA which is transcribed from a molecular marker when referring to protein products of a molecular marker). RNA or protein which
5 corresponds to a molecular marker are also considered the product of the molecular marker. In a specific embodiment, the level of an RNA product is measured using a technique which permits generation of data for a large number of molecular markers. However measured, the result is either the absolute or relative amounts of abundance of nucleic acids corresponding to the molecular markers, including but not limited to values
10 representing abundances or abundance ratios.

5.3.1.1 MICROARRAYS

In one embodiment, nucleic acid arrays are employed for analyzing the level of RNA product of each molecular marker of the genome in a blood sample. In a specific embodiment, molecular marker data is obtained by hybridizing detectably labeled
15 polynucleotides representing the nucleic acid sequences in mRNA transcripts present in a cell (*e.g.*, fluorescently labeled cDNA synthesized from total cell mRNA) to a microarray. In some embodiments expressed transcripts that may or may not represent genes expressed in the blood sample are analyzed.

In some embodiments, a microarray is an array of positionally-addressable binding
20 (*e.g.*, hybridization) sites on a support for representing many of the nucleic acid sequences in the genome of a cell or organism. In some embodiments, a microarray represents most or almost all of the genes in a species. In some embodiments, each microarray binding site consists of polynucleotide probes bound to a predetermined region on the support. Microarrays are described in Draghici, *Data Analysis Tools For DNA Microarrays*, 2003,
25 Chapman & Hall, CRC Press, New York, pp. 15-16, which is hereby incorporated by reference in its entirety.

Microarrays can be made in a number of ways. See, for example, Draghici, *Data Analysis Tools For DNA Microarrays*, 2003, Chapman & Hall, CRC Press, New York, pp. 16-22, which is hereby incorporated by reference in its entirety. Preferably microarrays are
30 reproducible, allowing multiple copies of a given array to be produced and results from the microarrays compared with each other. Preferably, the microarrays are made from materials that are stable under binding (*e.g.*, nucleic acid hybridization) conditions. Microarrays are preferably small, *e.g.*, between 1 cm² and 25 cm², preferably 1 to 3 cm². However, both larger and smaller arrays are also contemplated and may be preferable, *e.g.*,

for simultaneously evaluating a very large number or very small number of different probes.

In some embodiments, a given binding site or unique set of binding sites in the microarray will specifically bind (*e.g.*, hybridize) to a nucleotide sequence in a single gene
5 from a cell or organism (*e.g.*, to exon of a specific mRNA or a specific cDNA derived therefrom).

Microarrays used in the present invention can include one or more test probes. In some embodiments each such test probe comprises a polynucleotide sequence that is complementary to a subsequence of RNA or DNA to be detected. Each probe typically
10 has a different nucleic acid sequence, and the position of each probe on the solid surface of the array is usually known or can be determined. Microarrays useful in accordance with the invention can include oligonucleotide microarrays, cDNA based arrays, SNP arrays, spliced variant arrays and any other array able to provide a quantitative or semi quantitative data of the invention. Some types of microarrays are addressable arrays.
15 More specifically, some microarrays are positionally addressable arrays. In some embodiments, each probe of the array is located at a known, predetermined position on the solid support so that the identity (*e.g.*, the sequence) of each probe can be determined from its position on the array (*e.g.*, on the support or surface). In some embodiments, the arrays are ordered arrays.

20 In some embodiments, the density of probes on a microarray or a set of microarrays is 100 different (*e.g.*, non-identical) probes per 1 cm² or higher. More preferably, a microarray used in the methods of the invention will have at least 550 probes per 1 cm², at least 1,000 probes per 1 cm², at least 1,500 probes per 1 cm² or at least 2,000 probes per 1 cm². In a particularly preferred embodiment, the microarray is a high density array,
25 preferably having a density of at least 2,500 different probes per 1 cm². The microarrays used in the invention therefore preferably contain at least 2,500, at least 5,000, at least 10,000, at least 15,000, at least 20,000, at least 25,000, at least 50,000 or at least 55,000 different (*i.e.*, non-identical) probes.

In one embodiment, the microarray is an array (*e.g.*, a matrix) in which each
30 position represents a discrete binding site for a nucleotide sequence of a transcript encoded by a gene (*e.g.*, for an exon of an mRNA or a cDNA derived therefrom). The collection of binding sites on a microarray contains sets of binding sites for a plurality of genes and thus can be used, in some embodiments to measure molecular marker data. For example, in various embodiments, the microarrays of the invention can comprise binding sites for

products encoded by fewer than 50% of the genes in the genome of an organism.

Alternatively, the microarrays of the invention can have binding sites for the products encoded by at least 50%, at least 75%, at least 85%, at least 90%, at least 95%, at least 99% or 100% of the molecular markers of an organism. In other embodiments, the

5 microarrays of the invention can have binding sites for products encoded by fewer than 50%, by at least 50%, by at least 75%, by at least 85%, by at least 90%, by at least 95%, by at least 99% or by 100% of the genes expressed by a cell of an organism. The binding site can be a DNA or DNA analog to which a particular RNA can specifically hybridize. The DNA or DNA analog can be, e.g., a synthetic oligomer or a gene fragment, e.g.
10 corresponding to an exon.

In some embodiments of the present invention, an expressed transcript is represented in the nucleic acid arrays. In such embodiments, a set of binding sites can include probes with different polynucleotides that are complementary to different sequence segments of the expressed transcript. Exemplary polynucleotides that fall within this class
15 can be of length of 15 to 200 bases, 20 to 100 bases, 40-60 bases or some other range of bases. Each probe sequence can also comprise linker sequences in addition to the sequence that is complementary to its target sequence. As used herein, a linker sequence is a sequence between the sequence that is complementary to its target sequence and the surface of support. For example, in some embodiments, the nucleic acid arrays of the
20 invention comprise one probe specific to each target gene or exon. However, if desired, the nucleic acid arrays can contain at least 2, 5, 10, 100, or 1000 or more probes specific to some expressed transcript. For example, the array may contain probes tiled across the sequence of the longest mRNA isoform of a gene at single base steps.

In specific embodiments of the invention, when an exon has alternative spliced
25 variants, a set of polynucleotide probes of successive overlapping sequences, i.e., tiled sequences, across the genomic region containing the longest variant of an exon can be included in the exon nucleic acid arrays. The set of polynucleotide probes can comprise successive overlapping sequences at steps of a predetermined base intervals, e.g. at steps of 1, 5, or 10 base intervals, span, or are tiled across, the mRNA containing the longest
30 variant. Such sets of probes therefore can be used to scan the genomic region containing all variants of an exon to determine the expressed variant or variants of the exon to determine the expressed variant or variants of the exon. Alternatively or additionally, a set of polynucleotide probes comprising exon specific probes and/or variant junction probes can be included in the exon profiling array. As used herein, a variant junction probe refers

to a probe specific to the junction region of the particular exon variant and the neighboring exon. In some cases, the probe set contains variant junction probes specifically hybridizable to each of all different splice junction sequences of the exon. In other cases, the probe set contains exon specific probes specifically hybridizable to the common
5 sequences in all different variants of the exon, and/or variant junction probes specifically hybridizable to the different splice junction sequences of the exon.

In some cases, an exon is represented in the exon nucleic acid arrays by a probe comprising a polynucleotide that is complementary to the full length exon. In such instances, an exon is represented by a single binding site on the profiling arrays. In some
10 preferred cases, an exon is represented by one or more binding sites on the profiling arrays, each of the binding sites comprising a probe with a polynucleotide sequence that is complementary to an RNA fragment that is a substantial portion of the target exon. The lengths of such probes are normally between 15-600 bases, preferably between 20-200 bases, more preferably between 30-100 bases, and most preferably between 40-80 bases.
15 The average length of an exon is 200 bases (see, e.g., Lewin, Genes V, Oxford University Press, Oxford, 1994). A probe of length of 40-80 allows more specific binding of the exon than a probe of shorter length, thereby increasing the specificity of the probe to the target exon. For certain genes, one or more targeted exons may have sequence lengths less than 40-80 bases. In such cases, if probes with sequences longer than the target exons are to be
20 used, it may be desirable to design probes comprising sequences that include the entire target exon flanked by sequences from the adjacent constitutively splice exon or exons such that the probe sequences are complementary to the corresponding sequence segments in the mRNAs. Using flanking sequence from adjacent constitutively spliced exon or exons rather than the genomic flanking sequences, i.e., intron sequences, permits
25 comparable hybridization stringency with other probes of the same length. Preferably the flanking sequence used are from the adjacent constitutively spliced exon or exons that are not involved in any alternative pathways. More preferably the flanking sequences used do not comprise a significant portion of the sequence of the adjacent exon or exons so that cross-hybridization can be minimized. In some embodiments, when a target exon that is
30 shorter than the desired probe length is involved in alternative splicing, probes comprising flanking sequences in different alternatively spliced mRNAs are designed so that expression level of the exon expressed in different alternatively spliced mRNAs can be measured.

In some instances, when alternative splicing pathways and/or exon duplication in separate genes are to be distinguished, the DNA array or set of arrays can also comprise probes that are complementary to sequences spanning the junction regions of two adjacent exons. Preferably, such probes comprise sequences from the two exons which are not substantially overlapped with probes for each individual exons so that cross hybridization can be minimized. Probes that comprise sequences from more than one exons are useful in distinguishing alternative splicing pathways and/or expression of duplicated exons in separate genes if the exons occurs in one or more alternative spliced mRNAs and/or one or more separated genes that contain the duplicated exons but not in other alternatively spliced mRNAs and/or other genes that contain the duplicated exons. Alternatively, for duplicate exons in separate genes, if the exons from different genes show substantial difference in sequence homology, it is preferable to include probes that are different so that the exons from different genes can be distinguished.

It will be apparent to one skilled in the art that any of the probe schemes, supra, can be combined on the same nucleic acid array and/or on different arrays within the same set of nucleic acid arrays so that a more accurate determination of the expression profile for a plurality of molecular marker products can be accomplished. It will also be apparent to one skilled in the art that the different probe schemes can also be used for different levels of accuracies in profiling. For example, a nucleic acid array or array set comprising a small set of probes for each expressed transcript or each region thereof may be used to identify molecular markers under certain specific conditions. An array or array set comprising larger sets of probes for the exons that are of interest is then used to more accurately determine the specific molecular marker products under such specific conditions. Other DNA array strategies that allow more advantageous use of different probe schemes are also encompassed.

In some embodiments, the microarrays used in the invention can include binding sites (*e.g.*, probes) for sets of exons for one or more genes relevant to the condition of interest. The number of genes in a genome can be estimated from the number of mRNAs expressed by the cell or organism, or by extrapolation of a well characterized portion of the genome. When the genome of the organism of interest has been sequenced, the number of ORFs can be determined and mRNA coding regions identified by analysis of the DNA sequence. For example, the human genome is now known. Genome sequences for other organisms are also completed or nearly completed. Thus, in some embodiments of the invention, an array set comprising the total probes for all known or predicted exons

in the genome of an organism is provided. As a non-limiting example, the present invention provides an array set comprising one or two probes for each known or predicted exon of a molecular marker of the human genome.

It will be appreciated that when cDNA complementary to the RNA of a cell is made and hybridized to a microarray under suitable hybridization conditions, the level of hybridization to the site in the array corresponding to an exon of any particular molecular marker will reflect the prevalence in the cell of mRNA or mRNAs containing the exon transcribed from that molecular marker. For example, when detectably labeled (*e.g.*, with a fluorophore) cDNA complementary to the total cellular mRNA is hybridized to a microarray, the site on the array corresponding to an exon of a gene (*i.e.*, capable of specifically binding the product or products of the gene expressing) that is not transcribed or is removed during RNA splicing in the cell will have little or no signal (*e.g.*, fluorescent signal), and an exon of a gene for which the encoded mRNA expressing the exon is prevalent will have a relatively strong signal. The relative abundance of different mRNAs produced from the same gene by alternative splicing is then determined by the signal strength pattern across the whole set of exons monitored for the gene.

The use of a two-color fluorescence labeling and detection scheme to define alterations in gene expression has been described in connection with detection of mRNAs, *e.g.*, in Shena *et al.*, 1995, Science 270:467-470, which is incorporated by reference in its entirety for all purposes. In some embodiments, such schemes are used to measure molecular marker data. Such schemes are equally applicable to labeling and detection of expressed transcripts. An advantage of using cDNA labeled with two different fluorophores is that a direct and internally controlled comparison of the mRNA or exon expression levels corresponding to each arrayed gene in two blood samples can be made, and variations due to minor differences in experimental conditions (*e.g.*, hybridization conditions) will not affect subsequent analyses. However, it will be recognized that it is also possible to use cDNA from a single cell, and compare, for example, the absolute amount of a particular exon in, *e.g.*, a drug-treated or pathway-perturbed cell and an untreated cell. Furthermore, labeling with more than two colors is also contemplated in the present invention. In some embodiments of the invention, at least 5, 10, 20, or 100 dyes of different colors can be used for labeling. Such labeling permits simultaneous hybridizing of the distinguishably labeled cDNA populations to the same array, and thus measuring, and optionally comparing the expression levels of, mRNA molecules derived from more than two samples. Dyes that can be used include, but are not limited to,

fluorescein and its derivatives, rhodamine and its derivatives, texas red, 5'carboxy-fluorescein ("FMA"), 2',7'-dimethoxy-4',5'-dichloro-6-carboxy-fluorescein ("JOE"), N,N,N',N'-tetramethyl-6-carboxy-rhodamine ("TAMRA"), 6'carboxy-X-rhodamine ("ROX"), HEX, TET, IRD40, and IRD41, cyamine dyes, including but are not limited to
5 Cy3, Cy3.5 and Cy5; BODIPY dyes including but are not limited to BODIPY-FL, BODIPY-TR, BODIPY-TMR, BODIPY-630/650, and BODIPY-650/670; and ALEXA dyes, including but are not limited to ALEXA-488, ALEXA-532, ALEXA-546, ALEXA-568, and ALEXA-594; as well as other fluorescent dyes which will be known to those who are skilled in the art.

10 In one embodiment, hybridization levels at different hybridization times are measured separately on different, identical microarrays. For each such measurement, at hybridization time when hybridization level is measured, the microarray is washed briefly, preferably in room temperature in an aqueous solution of high to moderate salt concentration (e.g., 0.5 to 3 M salt concentration) under conditions which retain all bound
15 or hybridized polynucleotides while removing all unbound polynucleotides. The detectable label on the remaining, hybridized polynucleotide molecules on each probe is then measured by a method which is appropriate to the particular labeling method used. The resulted hybridization levels are then combined to form a hybridization curve. In another embodiment, hybridization levels are measured in real time using a single
20 microarray. In this embodiment, the microarray is allowed to hybridize to the sample without interruption and the microarray is interrogated at each hybridization time in a non-invasive manner. In still another embodiment, one can use one array, hybridize for a short time, wash and measure the hybridization level, put back to the same sample, hybridize for another period of time, wash and measure again to get the hybridization time curve.

25 In a specific embodiment, the Affymetrix® Human Genome U133 (HG-U133) Set, consisting of two GeneChip® arrays, is used in accordance with known methods. The Human Genome U133 (HG-U133) Set contains almost 45,000 probe sets representing more than 39,000 transcripts derived from approximately 33,000 well-substantiated human genes. This set design uses sequences selected from GenBank®, dbEST, and RefSeq. The
30 sequence clusters were created from the UniGene database (Build 133, April 20, 2001). They were then refined by analysis and comparison with a number of other publicly available databases including the Washington University EST trace repository and the University of California, Santa Cruz Golden Path human genome database (April 2001 release).

In another embodiment, the HG-U1 33A array is used in accordance with the methods of the invention. The HG-U133A array includes representation of the RefSeq database sequences and probe sets related to sequences previously represented on the Human Genome U95Av2 array. The HG-U1 33B array contains primarily probe sets
5 representing EST clusters. In another embodiment, the U133 Plus 2.0 GeneChip® is used in the invention. The U133 Plus 2.0 GeneChip® represents over 47,000 transcripts.

In another embodiment, a cDNA based microarray is used. In one embodiment the ChondroChip™ is used in accordance with the methods of the invention. The ChondroChip™ is a cDNA based microarray. One version of the ChondroChip™ includes
10 14,976 distinct elements: 10,382 known genes (69%), 4,112 EST/genomic DNA matches (28%), 328 clones with no significant match (2.2%), and 154 control spots (1.0%). Most if not all of the elements on the ChondroChip™ are complementary to ESTs identified as expressed in human chondrocytes. An article that describes the creation of a version of the ChondroChip™ is Zhang *et al*, 2002, Osteoarthritis and Cartilage 10, 950-960, which is
15 hereby incorporated by reference in its entirety.

In another embodiment, the BloodChip™ is used in accordance with the methods of the invention. The BloodChip is a cDNA microarray slide with 10,368 PCR products derived from peripheral blood cell cDNA libraries. The creation of the BloodChip™ microarray is described in Ma and Liew, 2003, Journal of Molecular and Cellular
20 Cardiology 8, 993-998, which is hereby incorporated by reference in its entirety.

5.3.1.2 TARGET POLYNUCLEOTIDE MOLECULES

Target polynucleotides that can be analyzed by the methods and compositions of the invention include RNA molecules such as, but by no means limited to, expressed RNA molecules which includes messenger RNA (mRNA) molecules, mRNA spliced variants as
25 well as other regulatory RNA, cRNA molecules (e.g., RNA molecules prepared from cDNA molecules that are transcribed in vivo) and fragments thereof. Target polynucleotides which may also be analyzed by the methods and compositions of the present invention include, but are not limited to DNA molecules such as genomic DNA molecules, cDNA molecules, and fragments thereof including oligonucleotides, ESTs,
30 STSs, etc

The target polynucleotide molecules may be naturally occurring nucleic acid molecules such as genomic or extragenomic DNA molecules isolated from a blood sample, or RNA molecules, such as mRNA molecules, isolated from a blood sample. The sample of target polynucleotides can comprise, e.g., molecules of DNA, RNA, or copolymers of

DNA and RNA. In specific embodiments, the target polynucleotides of the invention will correspond to particular genes or to particular gene transcripts (e.g., to particular mRNA sequences expressed in specific cell types or to particular cDNA sequences derived from such mRNA sequences). The target polynucleotides may correspond to different exons of
5 the same gene, e.g., so that different splice variants of that gene may be detected and/or analyzed.

In specific embodiments, the target polynucleotides to be analyzed are prepared in vitro from nucleic acids extracted from a blood sample. For example, in one embodiment, RNA is extracted from a blood sample (e.g., total cellular RNA, poly(A)+ messenger
10 RNA, fraction thereof) and messenger RNA is purified from the total extracted RNA. Methods for preparing total and poly(A)+ RNA are well known in the art, and are described generally, e.g., in Sambrook, Fritsch & Maniatis, "Molecular Cloning: A Laboratory Manual (1982); "DNA Cloning: A Practical Approach," Volumes I and II (D.N. Glover ed. 1985). In one embodiment, RNA is extracted from a blood sample using
15 guanidinium thiocyanate lysis followed by CsCl centrifugation and an oligo dT purification (Chirgwin et al., 1979, Biochemistry 18:5294-5299). In another embodiment, RNA is extracted from a blood sample using guanidinium thiocyanate lysis followed by purification on RNeasy columns (Qiagen). cDNA is then synthesized from the purified mRNA using, e.g., oligo-dT or random primers. In specific embodiments, the target
20 polynucleotides are cRNA prepared from purified messenger RNA extracted from a blood sample. As used herein, cRNA is defined here as RNA complementary to the source RNA. The extracted RNAs can be amplified using a process in which doubled-stranded cDNAs are synthesized from the RNAs using a primer linked to an RNA polymerase promoter in a direction capable of directing transcription of anti-sense RNA. Anti-sense
25 RNAs or cRNAs are then transcribed from the second strand of the double-stranded cDNAs using an RNA polymerase (see, e.g., U.S. Patent Nos. 5,891,636, 5,716,785; 5,545,522 and 6,132,997; see also, U.S. Patent No. 6,271,002, and U.S. Provisional Patent Application Serial No. 60/253,641, filed on November 28, 2000, by Ziman et al.). Both oligo-dT primers (U.S. Patent Nos. 5,545,522 and 6,132,997) or random primers (U.S.
30 Provisional Patent Application Serial No. 60/253,641, filed on November 28, 2000, by Ziman et al.) that contain an RNA polymerase promoter or complement thereof can be used. In some embodiments the target polynucleotides are short and/or fragmented polynucleotide molecules which are representative of the original nucleic acid population of the blood sample.

The target polynucleotides to be analyzed by the methods and compositions of the invention can be detectably labeled. For example, cDNA can be labeled directly, *e.g.*, with nucleotide analogs, or indirectly, *e.g.*, by making a second, labeled cDNA strand using the first strand as a template. Alternatively, the double-stranded cDNA can be transcribed into
5 cRNA and labeled.

In some embodiments the detectable label is a fluorescent label, *e.g.*, by incorporation of nucleotide analogs. Other labels suitable for use in the present invention include, but are not limited to, biotin, imminobiotin, antigens, cofactors, dinitrophenol, lipoic acid, olefinic compounds, detectable polypeptides, electron rich molecules, enzymes
10 capable of generating a detectable signal by action upon a substrate, and radioactive isotopes. Suitable radioactive isotopes include ^{32}P , ^{35}S , ^{14}C , ^{15}N and ^{125}I . Fluorescent molecules suitable for the present invention include, but are not limited to, fluorescein and its derivatives, rhodamine and its derivatives, texas red, 5'carboxy-fluorescein ("FMA"), 2',7'-dimethoxy-4',5'-dichloro-6-carboxy-fluorescein ("JOE"), N,N,N',N'- tetramethyl-6-
15 carboxy-rhodamine ("TAMRA"), 6'carboxy-X-rhodamine ("ROX"), HEX, TET, IRD40, and IRD41. Fluorescent molecules that are suitable for the invention further include: cyamine dyes, including but not limited to Cy3, Cy3.5 and Cy5; BODIPY dyes including but not limited to BODIPY-FL, BODIPY-TR, BODIPY-TMR, BODIPY-630/650, and BODIPY-650/670; and ALEXA dyes, including but not limited to ALEXA-488, ALEXA-
20 532, ALEXA-546, ALEXA-568, and ALEXA-594; as well as other fluorescent dyes which will be known to those who are skilled in the art. Electron rich indicator molecules suitable for the present invention include, but are not limited to, ferritin, hemocyanin, and colloidal gold. Alternatively, in less some embodiments the target polynucleotides may be labeled by specifically complexing a first group to the polynucleotide. A second group,
25 covalently linked to an indicator molecules and which has an affinity for the first group, can be used to indirectly detect the target polynucleotide. In such an embodiment, compounds suitable for use as a first group include, but are not limited to, biotin and iminobiotin. Compounds suitable for use as a second group include, but are not limited to, avidin and streptavidin.

30 In a specific embodiment, the target polynucleotides are prepared as follows: 2 μg Oligo-dT primers are annealed to 2 μg of mRNA isolated from a blood sample of a patient in a total volume of 15 μl , by heating to 70°C for 10 min, and cooled on ice. The mRNA is reverse transcribed by incubating the sample at 42°C for 1.5-2 hours in a 100 μl volume containing a final concentration of 50mM Tris-HCl (pH 8.3), 75mM KCl, 3mM MgCl_2 ,

25mM DTT, 25mM unlabeled dNTPs, 400 units of Superscript II (200U/ μ L, Gibco BRL), and 15mM of Cy3 or Cy5 (Amersham). RNA is then degraded by addition of 15 μ L of 0.1N NaOH, and incubation at 70°C for 10 min. The reaction mixture is neutralized by addition of 15 μ L of 0.1N HCl, and the volume is brought to 500 μ L with TE (10mM Tris, 1mM EDTA), and 20 μ L of Cot1 human DNA (Gibco-BRL) is added.

The labeled target polynucleotide molecules are purified by centrifugation in a Centricon-30 micro-concentrator (Amicon). If two different target polynucleotide samples (e.g., two samples derived from a healthy patient vs. patient with a disease) are being analyzed and compared by hybridization to the same array, each target nucleic acid sample is labeled with a different fluorescent label (e.g., Cy3 and Cy5) and separately concentrated. The separately concentrated target nucleic acid samples (Cy3 and Cy5 labeled) are combined into a fresh centricon, washed with 500 μ L TE, and concentrated again to a volume of less than 7 μ L. 1 μ L of 1 μ g/ μ L polyA RNA (Sigma, #P9403) and 1 μ L of 1 μ g/ μ L tRNA (Gibco-BRL, #15401-011) is added and the volume is adjusted to 9.5 μ L with distilled water. For final target polynucleotide preparation 2.1 μ L 20XSSC (1.5M NaCl, 150mM NaCitrate (pH8.0)) and 0.35 μ L 10%SDS is added.

5.3.1.3 HYBRIDIZATION TO MICROARRAYS

In some embodiments, nucleic acid hybridization and wash conditions are chosen so that the polynucleotide molecules to be analyzed by the invention (e.g., "target polynucleotide molecules") specifically bind or specifically hybridize to the complementary polynucleotide sequences of the array, typically to a specific array site, where its complementary DNA is located.

Arrays containing double-stranded probe DNA situated thereon can be subjected to denaturing conditions to render the DNA single-stranded prior to contacting with the target polynucleotide molecules. Arrays containing single-stranded probe DNA (e.g., synthetic oligodeoxyribonucleic acids) may need to be denatured prior to contacting with the target polynucleotide molecules, e.g., to remove hairpins or dimers which form due to self complementary sequences.

Optimal hybridization conditions will depend on the length (e.g., oligomer versus polynucleotide greater than 200 bases) and type (e.g., RNA, or DNA) of probe and target nucleic acids. General parameters for specific (i.e., stringent) hybridization conditions for nucleic acids are described in Sambrook *et al*, (*supra*), and in Ausubel *et al*, 1987, *Current Protocols in Molecular Biology*, Greene Publishing and Wiley-Interscience, New York. When the cDNA microarrays of Schena *et al*. are used, typical hybridization

conditions are hybridization in 5 X SSC plus 0.2% SDS at 65 °C for four hours, followed by washes at 25°C in low stringency wash buffer (1 X SSC plus 0.2% SDS), followed by 10 minutes at 25°C in higher stringency wash buffer (0.1 X SSC plus 0.2% SDS) (Shena *et al.*, 1996, *Proc. Natl. Acad. Sci. U.S.A.* P3:10614). Useful hybridization conditions are also provided in, *e.g.*, Tijessen, 1993, *Hybridization With Nucleic Acid Probes*, Elsevier Science Publishers B.V. and Kricka, 1992, *Nonisotopic DNA Probe Techniques*, Academic Press, San Diego, CA.

Representative hybridization conditions for use with the screening and/or signaling chips in accordance with some embodiments of the present invention include hybridization at a temperature at or near the mean melting temperature of the probes (*e.g.*, within 5 °C, more typically within 2 °C) in 1 M NaCl, 50 mM MES buffer (pH 6.5), 0.5% sodium Sarcosine and 30% formamide.

In a specific embodiment, a labeled target polynucleotide molecules are denatured by heating for two minutes at 100°C, and incubated at 37°C for 20-30 min before being placed on a nucleic acid array under a 22mm x 22mm glass cover slip. Hybridization is carried out at 65°C for fourteen to eighteen hours in a custom slide chamber with humidity maintained by a small reservoir of 3XSSC. The array is washed by submersion and agitation for between two and five minutes in 2X SSC with 0.1%SDS, followed by 1X SSC, and 0.1X SSC. Finally, the array is dried by centrifugation for 2 min in a slide rack in a Beckman GS-6 tabletop centrifuge in Microplus carriers at 650 RPM for two minutes.

5.3.1.4 SIGNAL DETECTION AND DATA ANALYSIS

It will be appreciated that when target sequences, *e.g.*, cDNA or cRNA, complementary to the RNA of a blood sample is made and hybridized to a microarray under suitable hybridization conditions, the level of hybridization to the site in the array corresponding to an exon of any particular gene will reflect the prevalence in the cell of mRNA or mRNAs containing the exon transcribed from that gene. For example, when detectably labeled (*e.g.*, with a fluorophore) cDNA complementary to the total cellular mRNA is hybridized to a microarray, the site on the array corresponding to an exon of a gene (*i.e.*, capable of specifically binding the product or products of the gene expressing) that is not transcribed or is removed during RNA splicing in the cell will have little or no signal (*e.g.*, fluorescent signal), and an exon of a gene for which the encoded mRNA expressing the exon is prevalent will have a relatively strong signal. The relative abundance of different mRNAs produced from the same gene by alternative splicing is

then determined by the signal strength pattern across the whole set of exons monitored for the gene.

Generally, any form of image processing may be used to digitize the microarrays and thereby obtain high throughput data for molecular markers in the present invention.

5 For example, any of the image processing techniques described or referenced in Draghici, *Data Analysis Tools For DNA Microarrays*, 2003, Chapman & Hall, CRC Press, New York, pp. 33-58, which is hereby incorporated by reference in its entirety, can be used. In some embodiments, two-color fluorescence is used. The use of a two-color fluorescence labeling and detection scheme to define alterations in gene expression has been described
10 in connection with detection of mRNAs, *e.g.*, in Shena *et al.*, 1995, *Science* 270:467-470, which is hereby incorporated by reference in its entirety for all purposes. The scheme is equally applicable to labeling and detection of exons. An advantage of using target sequences, *e.g.*, cDNAs or cRNAs, labeled with two different fluorophores is that a direct and internally controlled comparison of the mRNA or exon expression levels
15 corresponding to each arrayed gene in two states can be made, and variations due to minor differences in experimental conditions (*e.g.*, hybridization conditions) will not affect subsequent analyses.

In a specific embodiment, the labeled probes are scanned using a GMS Scanner 418 and Scanalyzer software (Michael Eisen, Stanford University), followed by
20 GeneSpring software (Silicon Genetics, CA) analysis. In another embodiment, a GMS Scanner 428 and Jaguar software are used followed by GeneSpring software analysis. In some embodiments a normalization routine, such as any of the normalization routines described in Section 5.7, is used.

5.3.2 RT-PCR AND QUANTITATIVE-PCR

25 In one aspect of the invention, the abundance or level of expression of an RNA product of a molecular marker can be measured performing reverse transcription on the RNA from blood and subsequently amplifying the resulting product ("RT-PCR"). In another embodiment, the abundance or level of expression of RNA can be measured from a blood sample by using quantitative RT-PCR or real time PCR ("QRT-PCR") on cDNA
30 copy of RNA. Total RNA, or mRNA from a blood sample can be used as a template and a primer specific to the transcribed portion of a gene of the invention is used to initiate reverse transcription. Methods of reverse transcribing RNA into cDNA are well known and described in, for example, Sambrook *et al.*, 1989, *supra*. Primer design can be accomplished utilizing commercially available software (*e.g.*, Primer Designer 1.0,

academic software, etc.). The product of the reverse transcription is subsequently used as a template for PCR. In one embodiment, a one step process can be used for either the RT-PCR and/or the QRT-PCR (combining the reverse transcription and PCR in a single reaction). In another embodiment, a two step process can be used for either the RT-PCR
5 and/or the QRT-PCR (first doing the reverse transcription step and subsequently performing the PCR). In some embodiments, oligo(dT)-primed first strand cDNA synthesis is performed so as to specifically target the mRNA population (e.g. using the Applied Biosystems High Capacity cDNA Archive Kit (cat # 4322171), on a Perkin-Elmer DNA Thermal Cycler.

10 For quantitative RT-PCR, in some embodiments the reportable value is the Ct-value, which is the threshold cycle at which PCR is in the logarithmic phase. For each gene of interest in each RNA sample, a ΔCt value can be calculated by the formula: $\Delta Ct = (Ct, \text{target gene}) - (Ct, \beta\text{-actin})$. The ΔCt values from different groups of RNA samples can then be compared by the Mann-Whitney Rank Sum test.

15 In some embodiments, Quantitative RT-PCR can be done using probes including Taqman® probes (Perkin Elmer, Foster City, California). The probe is specific for the PCR product and has both a quencher and fluorescent reporter attached to the probe. Different fluorescent markers can be utilized. In some embodiments, multiple probes can be used in the quantitative RT-PCR process to allow for multiplexing reactions (e.g. allow
20 for measurement of two molecular markers in one reaction well or container). When using TaqMan® probes, Taq DNA polymerase is used which has 5'-to-3' exonuclease activity and thus will cleave of the fluorescent reporter of the probe, freeing the fluorescent molecular from the quencher molecule. Thus the emission of fluorescence is used to measure the amount of PCR product being made. Other probes are also useful for
25 quantitative RT-PCR including Molecular Beacons®.

Other known techniques for quantitative Rt-PCR is to use an intercalating dye such as the commercially available QuantiTect™ SYBR® Green PCR (Qiagen, Valencia California).

30 Additionally, other systems to quantitatively measure mRNA expression products are known including Scorpions® (Zeneca Limited) or Fluorescent Polarization Probes (see e.g. *Zeneca Limited, 6,007,984*) etc.

5.3.3 NUCLEASE PROTECTION ASSAYS

Nuclease protection assays (including both ribonuclease protection assays and S1 nuclease assays) can be used to detect and quantify specific products of molecular

markers. In nuclease protection assays, an antisense probe (labeled with, e.g., radiolabeled or nonisotopic) hybridizes in solution to an RNA sample. Following hybridization, single-stranded, unhybridized probe and RNA are degraded by nucleases. An acrylamide gel is used to separate the remaining protected fragments. Typically, solution hybridization is more efficient than membrane-based hybridization, and it can accommodate up to 100 µg of sample RNA, compared with the 20-30 µg maximum of blot hybridizations.

The ribonuclease protection assay, which is the most common type of nuclease protection assay, requires the use of RNA probes. Oligonucleotides and other single-stranded DNA probes can only be used in assays containing S1 nuclease. The single-stranded, antisense probe must typically be completely homologous to target RNA to prevent cleavage of the probe-target hybrid by nuclease.

5.3.4 MASS SPECTROMETRY

Mass spectrometry (e.g., electrospray ionization "ESI", matrix-assisted laser desorption-ionization "MALDI", and Fourier-transform ion cyclotron resonance "FT-ICR") can be used to measure data (e.g., mass, charge) of molecular markers in blood samples. Such molecular markers that can be characterized by mass spectrometry include but are not limited to, proteins, nucleic acids, carbohydrates, and other biological macromolecules. This section provides brief and non-limiting examples of mass spectrometry techniques that can be used to quantitatively characterize molecular markers.

MALDI uses a pulsed laser for desorption of the ions and a time-of-flight analyzer and has been used for the detection of noncovalent tRNA:amino-acyl-tRNA synthetase complexes. See, for example, Gruic-Sovulj et al., 1997, J. Biol. Chem. 272:32084. ESI mass spectrometry ("ESI-MS") has been used for studying non-covalent molecular interactions. ESI-MS generates molecular ions with little to no fragmentation. See, for example, Xavier et al., 2000, Trends Biotechnol. 18:349. Fourier-transform ion cyclotron resonance ("FT-ICR") mass spectrometry provides high-resolution spectra, isotope-resolved precursor ion selection, and accurate mass assignments. See, for example, Xavier et al., 2000, Trends Biotechnol. 18:349.

Tandem mass spectrometry is described in Link et al., 1999, Nat. Biotechnol. 17, 676-682; Washburn et al. 2001, Nat. Biotechnol. 19, 242; Gaven et al., 2002, Nature 415, 141; and Ho et al., 2002, Nature 418, 180). In the case of proteins from a blood sample, the proteins can first be digested into peptides using an enzyme such as trypsin and then subjected to liquid chromatography tandem mass spectrometry (MS/MS). Liquid chromatography provides an initial separation of the peptides, which are then ionized

directly into a mass spectrometer. Following an initial scan in which the mass/charge ratio of all intact (parent) ions from the peptides are measured, the mass spectrometer selects a parent ion, fragments it and obtains the mass spectrum of the generated fragments. These fragmentation patterns are called tandem mass spectra or MS/MS spectra. This process of ion selection and fragmentation is repeated throughout the liquid chromatography separation, thus generating a set of time resolved MS/MS spectra, with each spectrum representing a species eluting at a particular time from the LC separation. The resolving power of the liquid chromatography step, combined with the high mass resolution of modern mass spectrometers typically assures that each MS/MS spectrum represents the fragmentation pattern of a unique peptide in the digest.

5.3.5 COMPARATIVE GENE EXPRESSION PROFILING

In some embodiments of the present invention quantitative measurement of molecular marker data is performed using comparative gene-expression profiling. An example of such technology is the multiplex microsphere bead assay used by Fuja et al., 2004, Journal of Biotechnology 108, 193,.

5.3.6 TRANSCRIPTION BASED AMPLIFICATION SYSTEMS

In another aspect of the invention, the level of expression of a molecular marker in blood can be measured by amplifying RNA from a blood sample using transcription based amplification systems (TAS), including nucleic acid sequence amplification (NASBA) and 3SR. See, e.g., Kwoh et al., 1989, PNAS USA 86:1 173; International Publication No. WO 88/10315; and U.S. Patent No. 6,329,179. In NASBA, the nucleic acids can be prepared for amplification using conventional phenol/chloroform extraction, heat denaturation, treatment with lysis buffer and minispin columns for isolation of DNA and RNA or guanidinium chloride extraction of RNA. These amplification techniques involve annealing a primer that has target specific sequences. Following polymerization, DNA/RNA hybrids are digested with RNase H while double stranded DNA molecules are heat denatured again. In either case, the single stranded DNA is made fully double stranded by addition of second target specific primer, followed by polymerization. The double-stranded DNA molecules are then multiply transcribed by a polymerase such as T7 or SP6. In an isothermal cyclic reaction, the RNA's are reverse transcribed into double stranded DNA, and transcribed once with a polymerase such as T7 or SP6. The resulting products, whether truncated or complete, indicate target specific sequences.

5.3.7 ADDITIONAL TECHNIQUES FOR DETECTING AND QUANTIFYING RNA

Many other techniques are known to one of skill for detecting and measuring RNA
5 and can be used in accordance with the methods of the invention. Non-limiting examples
of such techniques include Northern blotting, nuclease protection assays, RNA
fingerprinting, polymerase chain reaction, ligase chain reaction, Qbeta replicase,
isothermal amplification method, strand displacement amplification, transcription based
amplification systems, nuclease protection (SI nuclease or RNase protection assays) as
10 well as methods disclosed in International Publication Nos. WO 88/10315 and WO
89/06700, and International Applications Nos. PCT/US87/00880 and PCT/US 89/01025.

A standard Northern blot assay can be used to ascertain an RNA transcript size,
identify alternatively spliced RNA transcripts, and the relative amounts of mRNA in a
blood sample, in accordance with conventional Northern hybridization techniques known
15 to those persons of ordinary skill in the art. In Northern blots, RNA samples are first
separated by size via electrophoresis in an agarose gel under denaturing conditions. The
RNA is then transferred to a membrane, crosslinked and hybridized with a labeled probe.
Nonisotopic or high specific activity radiolabeled probes can be used including random-
primed, nick-translated, or PCR-generated DNA probes, in vitro transcribed RNA probes,
20 and oligonucleotides. Additionally, sequences with only partial homology (e.g., cDNA
from a different species or genomic DNA fragments that might contain an exon) may be
used as probes. The labeled probe, e.g., a radiolabeled cDNA, either containing the full-
length, single stranded DNA or a fragment of that DNA sequence may be at least 20, at
least 30, at least 50, or at least 100 consecutive nucleotides in length. The probe can be
25 labeled by any of the many different methods known to those skilled in this art. The labels
most commonly employed for these studies are radioactive elements, enzymes, chemicals
that fluoresce when exposed to ultraviolet light, and others. A number of fluorescent
materials are known and can be utilized as labels. These include, but are not limited to,
fluorescein, rhodamine, auramine, Texas Red, AMCA blue and Lucifer Yellow. A
30 articular detecting material is anti-rabbit antibody prepared in goats and conjugated with
fluorescein through an isothiocyanate. Proteins can also be labeled with a radioactive
element or with an enzyme. The radioactive label can be detected by any of the currently
available counting procedures. Non-limiting examples of isotopes include ^3H , ^{14}C , ^{32}P ,
 ^{35}S , ^{36}Cl , ^{51}Cr , ^{57}Co , ^{58}Co , ^{59}Fe , ^{90}Y , ^{125}I , ^{131}I , and ^{186}Re . Enzyme labels are

likewise useful, and can be detected by any of the presently utilized colorimetric, spectrophotometric, fluorospectrophotometric, amperometric or gasometric techniques. The enzyme is conjugated to the selected particle by reaction with bridging molecules such as carbodiimides, diisocyanates, glutaraldehyde and the like. Any enzymes known to one
5 of skill in the art can be utilized. Examples of such enzymes include, but are not limited to, peroxidase, beta-D-galactosidase, urease, glucose oxidase plus peroxidase and alkaline phosphatase. U.S. Patent Nos. 3,654,090, 3,850,752, and 4,016,043 are referred to by way of example for their disclosure of alternate labeling material and methods.

Nuclease protection assays (including both ribonuclease protection assays and S1
10 nuclease assays) can be used to detect and quantitate specific mRNAs. In nuclease protection assays, an antisense probe (labeled with, e.g., radiolabeled or nonisotopic) hybridizes in solution to an RNA sample. Following hybridization, single-stranded, unhybridized probe and RNA are degraded by nucleases. An acrylamide gel is used to separate the remaining protected fragments. Typically, solution hybridization is more
15 efficient than membrane-based hybridization, and it can accommodate up to 100 µg of sample RNA, compared with the 20-30 µg maximum of blot hybridizations.

The ribonuclease protection assay, which is the most common type of nuclease protection assay, requires the use of RNA probes. Oligonucleotides and other single-stranded DNA probes can only be used in assays containing S1 nuclease. The single-
20 stranded, antisense probe must typically be completely homologous to target RNA to prevent cleavage of the probe:target hybrid by nuclease.

Additional techniques to quantitatively measure RNA expression include, but are not limited to, ligase chain reaction, Qbeta replicase (see, e.g., International Application No. PCT/US87/00880), isothermal amplification method (see, e.g., Walker et al., 1992,
25 PNAS 89:382-396), strand displacement amplification (SDA), repair chain reaction, Asymmetric Quantitative PCR (see, e.g., U.S. Publication No. US200330134307A1) and the multiplex microsphere bead assay described in Fuja et al., 2004, Journal of Biotechnology 108:193-205.

5.3.7.1 SEPARATION OF AMPLIFICATION PRODUCTS

30 Some of the quantitative measurement techniques described above may require separation of amplification products. Several techniques can be used to separate such amplification products. For example, amplification products can be separated by agarose, agarose-acrylamide or polyacrylamide gel electrophoresis using conventional methods. Several techniques for detecting PCR products quantitatively without electrophoresis can

also be used according to the invention. See, for example, *CR Protocols, A Guide to Methods and Applications*, Innis *et al*, Academic Press, Inc. N.Y., 1990). For example, chromatographic techniques can be employed to effect separation. There are many kinds of chromatography that can be used in the present invention: adsorption, partition, ion-
5 exchange and molecular sieve, HPLC, and many specialized techniques for using them including column, paper, thin-layer and gas chromatography (Freifelder, *Physical Biochemistry Applications to Biochemistry and Molecular Biology*, 2nd ed., Wm. Freeman and Co., New York, N.Y., 1982).

Another example of a separation methodology is done by covalently labeling the
10 oligonucleotide primers used in a PCR reaction with various types of small molecule ligands. In one such separation, a different ligand is present on each oligonucleotide. A molecule, perhaps an antibody or avidin if the ligand is biotin, that specifically binds to one of the ligands is used to coat the surface of a plate such as a 96 well ELISA plate. Upon application of the PCR reactions to the surface of such a prepared plate, the PCR
15 products are bound with specificity to the surface. After washing the plate to remove unbound reagents, a solution containing a second molecule that binds to the first ligand is added. This second molecule is linked to some kind of reporter system. The second molecule only binds to the plate if a PCR product has been produced whereby both oligonucleotide primers are incorporated into the final PCR products. The amount of the
20 PCR product is then detected and quantified in a commercial plate reader much as ELISA reactions are detected and quantified. An ELISA-like system such as the one described here has been developed by the Raggio Italgene company under the C-Track trade name.

5.3.7.2 VISUALIZATION OF AMPLIFICATION PRODUCTS

Some of the quantitative measurement techniques described above may require
25 visualization of amplification products. Amplification products are visualized, for example, in order to confirm amplification of the marker sequences. One typical visualization method involves staining of a gel with ethidium bromide and visualization under UV light. Alternatively, if the amplification products are integrally labeled with radio- or fluorometrically-labeled nucleotides, the amplification products may then be
30 exposed to x-ray film or visualized under the appropriate stimulating spectra, following separation.

In one embodiment, visualization is achieved indirectly. Following separation of amplification products, a labeled, nucleic acid probe is brought into contact with the amplified marker sequence. The probe preferably is conjugated to a chromophore but may

be radiolabeled. In another embodiment, the probe is conjugated to a binding partner, such as an antibody or biotin, where the other member of the binding pair carries a detectable moiety.

In another embodiment, detection is by Southern blotting and hybridization with a labeled probe. The techniques involved in Southern blotting are well known to those of skill in the art and may be found in many standard books on molecular protocols. See Sambrook et al., 1989. Briefly, amplification products are separated by gel electrophoresis. The gel is then contacted with a membrane, such as nitrocellulose, permitting transfer of the nucleic acid and non-covalent binding. Subsequently, the membrane is incubated with a chromophore-conjugated probe that is capable of hybridizing with a target amplification product. Detection is by exposure of the membrane to x-ray film or ion-emitting detection devices.

One example of the foregoing is described in U.S. Pat. No. 5,279,721, incorporated by reference herein, which discloses an apparatus and method for the automated electrophoresis and transfer of nucleic acids. The apparatus permits electrophoresis and blotting without external manipulation of the gel and is ideally suited to carrying out methods according to the present invention.

5.4 METHODS FOR MEASURING MOLECULAR MARKER DATA REFLECTIVE OF ABUNDANCE OF PROTEIN PRODUCTS OF MOLECULAR MARKERS

Measurement of the abundance of protein products of molecular markers in blood may be performed using a number of separation techniques combined with a monitoring system. For example, whole genome monitoring of protein (e.g., the "proteome,") can be carried out using commercial systems such as a SELDI® Chip by Ciphergen. In addition, protein microarrays comprised of immobilized, preferably monoclonal, antibodies specific to a plurality of protein species encoded by the cell genome can be used, (e.g., The ProteinChip® Biomarker System, Ciphergen, Fremont, California). See also, for example, Lin, 2004, *Modern Pathology*, 1-9; Li, 2004, *Journal of Urology* 171, 1782-1787; Wadsworth, 2004, *Clinical Cancer Research*, 10, 1625-1632; Prieto, 2003, *Journal of Liquid Chromatography & Related Technologies* 26, 2315-2328; Coombes, 2003, *Clinical Chemistry* 49, 1615-1623; Mian, 2003, *Proteomics* 3, 1725-1737; Lehre *et al*, 2003, *BJU International* 92, 223-225; and Diamond, 2003, *Journal of the American Society for Mass Spectrometry* 14, 760-765, which are hereby incorporated by reference in their entireties.

In one embodiment, antibodies can be used to measure protein products of the candidate molecular markers. Methods for making monoclonal antibodies are well known (see, e.g., Harlow and Lane, 1988, *Antibodies: A Laboratory Manual*, Cold Spring Harbor, New York, which is incorporated in its entirety for all purposes). In one embodiment, 5 monoclonal antibodies are raised against synthetic peptide fragments designed based on genomic sequence of the cell. With such an antibody array, proteins from the cell are contacted to the array and their binding is assayed with assays known in the art.

Immunoassays known to one of skill in the art can be used to detect and quantify protein levels. For example, ELISAs can be used to detect and quantify protein levels. 10 ELISAs comprise preparing antigen, coating the well of a 96 well microtiter plate with the antigen, adding the antibody of interest conjugated to a detectable compound such as an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) to the well and incubating for a period of time, and detecting the presence of the antigen. In ELISAs the antibody of interest does not have to be conjugated to a detectable compound; instead, a 15 second antibody (which recognizes the antibody of interest) conjugated to a detectable compound may be added to the well. Further, instead of coating the well with the antigen, the antibody may be coated to the well. In this case, a second antibody conjugated to a detectable compound may be added following the addition of the antigen of interest to the coated well. One of skill in the art would be knowledgeable as to the parameters that can 20 be modified to increase the signal detected as well as other variations of ELISAs known in the art. In a preferred embodiment, an ELISA may be performed by coating a high binding 96-well microtiter plate (Costar) with 2 µg/ml of rhu-IL-9 in PBS overnight. Following three washes with PBS, the plate is incubated with three-fold serial dilutions of Fab at 25°C for 1 hour. Following another three washes of PBS, 1 µg/ml anti-human 25 kappa-alkaline phosphatase-conjugate is added and the plate is incubated for 1 hour at 25°C. Following three washes with PBST, the alkaline phosphatase activity is determined in 50 µl/AMP/PPMP substrate. The reactions are stopped and the absorbance at 560 nm is determined with a VMAX microplate reader. For further discussion regarding ELISAs see, e.g., Ausubel et al, eds, 1994, *Current Protocols in Molecular Biology*, Vol. 1, John 30 Wiley & Sons, Inc., New York at 11.2.1.

Protein levels may be determined by Western blot analysis. Further, protein levels as well as the phosphorylation of proteins can be determined by immunoprecipitation followed by Western blot analysis. Immunoprecipitation protocols generally comprise lysing a population of cells in a lysis buffer such as RIPA buffer (1% NP-40 or Triton X-

100, 1% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 0.01 M sodium phosphate at pH 7.2, 1% Trasylol) supplemented with protein phosphatase and/or protease inhibitors (*e.g.*, EDTA, PMSF, aprotinin, sodium vanadate), adding the antibody of interest to the cell lysate, incubating for a period of time (*e.g.*, 1 to 4 hours) at 40° C, adding protein A and/or
5 protein G sepharose beads to the cell lysate, incubating for about an hour or more at 40° C, washing the beads in lysis buffer and resuspending the beads in SDS/sample buffer. The ability of the antibody of interest to immunoprecipitate a particular antigen can be assessed by, *e.g.*, western blot analysis. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the binding of the antibody to an antigen and
10 decrease the background (*e.g.*, pre-clearing the cell lysate with sepharose beads). For further discussion regarding immunoprecipitation protocols see, *e.g.*, Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.16.1.

Western blot analysis generally comprises preparing protein samples,
15 electrophoresis of the protein samples in a polyacrylamide gel (*e.g.*, 8%- 20% SDS-PAGE depending on the molecular weight of the antigen), transferring the protein sample from the polyacrylamide gel to a membrane such as nitrocellulose, PVDF or nylon, incubating the membrane in blocking solution (*e.g.*, PBS with 3% BSA or non-fat milk), washing the membrane in washing buffer (*e.g.*, PBS-Tween 20), incubating the membrane with
20 primary antibody (the antibody of interest) diluted in blocking buffer, washing the membrane in washing buffer, incubating the membrane with a secondary antibody (which recognizes the primary antibody, *e.g.*, an anti-human antibody) conjugated to an enzymatic substrate (*e.g.*, horseradish peroxidase or alkaline phosphatase) or radioactive molecule (*e.g.*, ³²p or ¹²⁵I) diluted in blocking buffer, washing the membrane in wash buffer, and
25 detecting the presence of the antigen. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected and to reduce the background noise. For further discussion regarding western blot protocols see, *e.g.*, Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.8.1.

30 Protein expression levels can also be separated by two-dimensional gel electrophoresis systems. Two-dimensional gel electrophoresis is well-known in the art and typically involves isoelectric focusing along a first dimension followed by SDS-PAGE electrophoresis along a second dimension. See, *e.g.*, Hames *et al*, 1990, *Gel Electrophoresis of Proteins: A Practical Approach*, IRL Press, New York; Shevchenko *et*

al., 1996, *Proc. Natl. Acad. Sci. USA* 93:1440-1445; Sagliocco *et al.*, 1996, *Yeast* 12:1519-1533; Lander, 1996, *Science* 274:536-539. The resulting electropherograms can be analyzed by numerous techniques, including mass spectrometric techniques, Western blotting and immunoblot analysis using polyclonal and monoclonal antibodies, and
5 internal and N-terminal micro-sequencing.

5.5 USES OF CLASSIFIERS IDENTIFIED

In exemplary embodiments, the classifiers constructed in accordance with the present invention can be used to detect, diagnose, prognose and/or monitor a trait in a test individual. In a specific embodiment, a classifier or classifier group constructed in
10 accordance with Section 5.1 is used to detect, diagnose, prognose and/or monitor a disease in said test individual. In another embodiment, a classifier or classifier group constructed in accordance with Section 5.1 is used to detect, diagnose, prognose and/or monitor a reoccurrence of disease in said test individual. In another embodiment, a classifier or classifier group constructed in accordance with the invention is used to evaluate or predict
15 the efficacy of treatment in a subject. In another embodiment, a classifier or classifier group constructed in accordance with the invention is used to predict whether a subject will be responsive to treatment and/or treatment outcomes. In another embodiment, a classifier or classifier group constructed in accordance with the invention is used to monitor and/or predict treatment compliance or non-compliance. In another embodiment,
20 a classifier or classifier group constructed in accordance with the methods of the invention is indicative of the responsiveness of a subject to a stimulus (whether external or internal, e.g., smoke, pollution, sunlight, heat, and mutations) and is used to evaluate or predict the response of a subject to such stimulus.

In yet another embodiment, the molecular markers identified by the classifiers of
25 the invention can be used independently of the classifier to detect, diagnose, prognose, predict and/or monitor a trait. In such embodiments, said detection, diagnosis, prognosis, prediction and/or monitoring of a test individual can be accomplished by monitoring the gene expression pattern or profile of the molecular markers identified by the classifier or classifier group of the invention of a test individual and comparing said pattern or profile
30 to a gene expression pattern or profile of a control individual or group of individuals who have said trait. In another embodiment, the gene expression pattern or profile of the test individual can be compared with a control individual or group of individuals who do not have said trait. In another embodiment, the gene expression pattern or profile of the test individual is compared as between individuals or group of individuals who have said trait

and who do not have said trait. In yet another embodiment, the gene expression pattern or profile of the test individual is compared with the individuals used for the training population. In yet another embodiment, the gene expression pattern or profile of the test individual is compared with the individuals of the scoring population. As used herein, a

5 "gene expression pattern" or "gene expression profile" indicates the combined pattern of the results of the analysis of the level of expression of two or more biomarkers of the invention including 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or all of the biomarkers of the classifier or classifier groups. A gene expression pattern or gene expression profile can result from the measurement of expression of the products of the biomarkers of the invention and can be

10 done by measuring either the RNA or the proteins corresponding to said molecular marker using any any of the techniques described herein. For example techniques to measure expression of the RNA products of the biomarkers of the invention includes, PCR based methods (including RT-PCR and quantitative RT-PCR) and non PCR based method ' as well as microarray analysis. To measure protein products of the biomarkers of the

15 invention, techniques include western blotting and ELISA analysis.

5.6 KITS

One embodiment of the present invention comprises kits for measuring molecular marker data by providing the materials necessary to measure the abundance of one or more of the products of one or more molecular markers of the classifier or classifier groups

20 identified. Such kits may comprise materials and reagents required for measuring molecular marker data where the product of the molecular marker is RNA or protein. In some embodiments, such kits include microarrays wherein the microarray is comprised of oligonucleotides and/or DNA and/or RNA fragments which hybridize to one or more of the products of one or more of the molecular markers of a classifier or classifier group. In

25 some embodiments, such kits may include primers for PCR of either the RNA product or the cDNA copy of the RNA product of the molecular marker or both. In some embodiments, such kits may include primers for PCR as well as probes for Quantitative PCR. In some embodiments, such kits may include multiple primers and multiple probes wherein some of said probes have different flourophores so as to permit multiplexing of

30 multiple products of a single molecular marker or multiple products wherein each product results from a single molecular marker. In some embodiments, such kits may further include materials and reagents for creating cDNA from RNA. In some embodiments, such kits may include antibodies specific for the protein products of a molecular marker. Such kits may additionally comprise materials and reagents for isolating RNA and/or proteins

from a blood sample. Such kits may additionally comprise materials and reagents for isolating RNA and/or proteins from a non-blood tissue sample. In addition such kits may include materials and reagents for synthesizing cDNA from RNA isolated from a blood sample. In some embodiments of the present invention such kits may include, a computer
5 program product embedded on computer readable media for determining whether a subject has a trait of interest. In some embodiments of the present invention, the kits of the invention may include a computer program product embedded on a computer readable media along with instructions.

In some embodiments, the invention provides kits for measuring the expression of
10 one or more nucleic acid sequences of one or more molecular markers. In a specific embodiment, such kits measure the expression of one or more nucleic acid sequences associated with a molecular marker which has been determined according to the method of the invention as being indicative of a trait of interest, in accordance with this embodiment, the kits may comprise materials and reagents that are necessary for
15 measuring the expression of particular nucleic acid sequence products of molecular markers identified by a classifier or classifier group of the invention. For example, a microarray or RT-PCR kit may be produced for a specific condition and contain only those reagents and materials necessary for measuring the levels of specific RNA transcript products of the molecular markers associated with the classifier or classifier groups
20 selected in accordance with one embodiment of the invention. Alternatively, in some embodiments, the kits can comprise materials and reagents that are not limited to those required to measure the expression of particular nucleic acid sequences of any particular molecular marker. For example, in certain embodiments, the kits comprise materials and reagents necessary for measuring the levels of expression of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15,
25 20, 25, 30, 35, 40, 45, 50 or more of the molecular markers of the invention, in addition to reagents and materials necessary for measuring the levels of the expression of at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50 or more genes other than the molecular markers of the invention. In other embodiments,
30 the kits contain reagents and materials necessary for measuring the levels of expression of at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50 or more of the molecular markers of the invention, and 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 125, 150, 175, 200, 225, 250,

300, 350, 400, 450, or more genes that are not molecular markers of the invention, or 1-10, 1-100, 1-150, 1-200, 1-300, 1-400, 1-500, 1-1000, 25-100, 25-200, 25-300, 25-400, 25-500, 25-1000, 100-150, 100-200, 100-300, 100-400, 100-500, 100-1000 or 500-1000 genes that are not molecular markers of the invention.

5 For nucleic acid microarray kits, the kits generally comprise probes attached to a solid support surface. In one such embodiment, probes can be either oligonucleotides or longer length probes including probes ranging from 150 nucleotides in length to 800 nucleotides in length. The probes may be labeled with a detectable label. In a specific embodiment, the probes are specific for one or more of the products of a specific molecular marker identified following the methods of section 5.1. The microarray kits
10 may comprise instructions for performing the assay and methods for interpreting and analyzing the data resulting from the performance of the assay. In a specific embodiment, the kits comprise instructions for diagnosing a trait of interest. The kits may also comprise hybridization reagents and/or reagents necessary for detecting a signal produced when a
15 probe hybridizes to a target nucleic acid sequence. Generally, the materials and reagents for the microarray kits are in one or more containers. Each component of the kit is generally in its own a suitable container.

In certain embodiments, a nucleic acid microarray kit comprises materials and reagents necessary for measuring the levels of expression of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15,
20 20, 25, 30, 35, 40, 45, 50 or more of the molecular markers of the invention, in addition to reagents and materials necessary for measuring the levels of the expression of at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50 or more genes other than the molecular markers of the invention. In other embodiments, a
25 nucleic acid microarray kit contains reagents and materials necessary for measuring the levels of expression of at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50 or more of the molecular markers of the invention, and 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 125,
30 150, 175, 200, 225, 250, 300, 350, 400, 450, or more genes that are not molecular markers of the invention, or 1-10, 1-100, 1-150, 1-200, 1-300, 1-400, 1-500, 1-1000, 25-100, 25-200, 25-300, 25-400, 25-500, 25-1000, 100-150, 100-200, 100-300, 100-400, 100-500, 100-1000 or 500-1000 genes that are not molecular markers of the invention.

For Quantitative PCR, the kits generally comprise pre-selected primers specific for particular nucleic acid sequences. The Quantitative PCR kits may also comprise enzymes suitable for amplifying nucleic acids (e.g., polymerases such as Taq), and deoxynucleotides and buffers needed for the reaction mixture for amplification. The

5 Quantitative PCR kits may also comprise probes specific for the nucleic acid sequences associated with or indicative of a condition. The probes may or may not be labeled with a fluorophore. The probes may or may not be labeled with a quencher molecule. In some embodiments the Quantitative PCR kits also comprise components suitable for reverse-transcribing RNA including enzymes (e.g. reverse transcriptases such as AMV, MMLV

10 and the like) and primers for reverse transcription along with deoxynucleotides and buffers needed for the reverse transcription reaction. Each component of the quantitative PCR kit is generally in its own suitable container. Thus, these kits generally comprise distinct containers suitable for each individual reagent, enzyme, primer and probe. Further, the quantitative PCR kits may comprise instructions for performing the assay and methods for

15 interpreting and analyzing the data resulting from the performance of the assay. In a specific embodiment, the kits contain instructions for diagnosing a trait of interest.

For antibody based kits, the kit can comprise, for example: (1) a first antibody (which may or may not be attached to a solid support) which binds to a peptide, polypeptide or protein of interest; and, optionally, (2) a second, different antibody which

20 binds to either the peptide, polypeptide or protein, or the first antibody and is conjugated to a detectable label (e.g., a fluorescent label, radioactive isotope or enzyme). In a specific embodiment, the peptide, polypeptide or protein of interest is associated with or indicative of a condition (e.g., a disease). The antibody-based kits may also comprise beads for conducting an immunoprecipitation. Each component of the antibody-based kits is

25 generally in its own suitable container. Thus, these kits generally comprise distinct containers suitable for each antibody. Further, the antibody-based kits may comprise instructions for performing the assay and methods for interpreting and analyzing the data resulting from the performance of the assay. In a specific embodiment, the kits contain instructions for diagnosing a trait of interest.

30 5.7 EXEMPLARY NORMALIZATION ROUTINES

A number of different normalization protocols can be used to normalize molecular marker data obtained using microarrays. Some such normalization protocols are described in this section. Typically, the normalization comprises normalizing the expression level measurement of each gene in a plurality of genes that is expressed by a subject. Many of

the normalization protocols described in this section are used to normalize microarray data. It will be appreciated that there are many other suitable normalization protocols that may be used in accordance with the present invention. All such protocols are within the scope of the present invention. Many of the normalization protocols found in this section are found in publicly available software, such as Microarray Explorer (Image Processing Section, Laboratory of Experimental and Computational Biology, National Cancer Institute, Frederick, MD 21702, USA).

One normalization protocol is Z-score of intensity. In this protocol, raw expression intensities are normalized by the (mean intensity)/(standard deviation) of raw intensities for all spots in a sample. For microarray data, the Z-score of intensity method normalizes each hybridized sample by the mean and standard deviation of the raw intensities for all of the spots in that sample. The mean intensity m_{li} and the standard deviation s_{li} are computed for the raw intensity of control genes. It is useful for standardizing the mean (to 0.0) and the range of data between hybridized samples to about -3.0 to +3.0. When using the Z-score, the Z differences (Z_{dif}) are computed rather than ratios. The Z-score intensity ($Z\text{-score}_{ij}$) for intensity I_{ij} for probe i (hybridization probe, protein, or other binding entity) and spot j is computed as:

$$Z\text{-score}_{ij} = (I_{ij} - m_{li}) / s_{li},$$

and

$$Z_{diff}(x,y) = Z\text{-score}_x - Z\text{-score}_y,$$

where x represents the x channel and y represents the y channel.

Another normalization protocol is the median intensity normalization protocol in which the raw intensities for all spots in each sample are normalized by the median of the raw intensities. For microarray data, the median intensity normalization method normalizes each hybridized sample by the median of the raw intensities of control genes ($median_{li}$) for all of the spots in that sample. Thus, upon normalization by the median intensity normalization method, the raw intensity I_{ij} for probe i and spot j , has the value I_{ij} where,

$$I_{ij} = (I_{ij} / median_{li}).$$

Another normalization protocol is the log median intensity protocol. In this protocol, raw expression intensities are normalized by the log of the median scaled raw intensities of representative spots for all spots in the sample. For microarray data, the log

median intensity method normalizes each hybridized sample by the log of median scaled raw intensities of control genes (median_{li}) for all of the spots in that sample. As used herein, control genes are a set of genes that have reproducibly accurately measured expression values. The value 1.0 is added to the intensity value to avoid taking the
 5 log(O.O) when intensity has zero value. Upon normalization by the median intensity normalization method, the raw intensity I_{ij} for probe i and spot j , has the value I_{mj} where,

$$I_{mj} = \log(1.0 + (I_{ij} / \text{median}_{li})).$$

Yet another normalization protocol is the Z-score standard deviation log of
 10 intensity protocol. In this protocol, raw expression intensities are normalized by the mean log intensity (mnLI_j) and standard deviation log intensity (sdLI_j). For microarray data, the mean log intensity and the standard deviation log intensity is computed for the log of raw intensity of control genes. Then, the Z-score intensity ZlogS_j for probe i and spot j is:

$$ZlogS_{j_i} = (\log(I_{ij}) - \text{mnLI}_i) / \text{sdLI}_i.$$

15 Still another normalization protocol is the Z-score mean absolute deviation of log intensity protocol. In this protocol, raw expression intensities are normalized by the Z-score of the log intensity using the equation (log(intensity)-mean logarithm) / standard deviation logarithm. For microarray data, the Z-score mean absolute deviation of log intensity protocol normalizes each bound sample by the mean and mean absolute deviation
 20 of the logs of the raw intensities for all of the spots in the sample. The mean log intensity mnLI_j and the mean absolute deviation log intensity madLI_j are computed for the log of raw intensity of control genes. Then, the Z-score intensity ZlogA_j for probe i and spot j is:

$$ZlogA_{jj} = (\log(I_{ij}) - \text{mnLI}_i) / \text{madLI}_i.$$

Another normalization protocol is the user normalization gene set protocol. In this
 25 protocol, raw expression intensities are normalized by the sum of the genes in a user defined gene set in each sample. This method is useful if a subset of genes has been determined to have relatively constant expression across a set of samples. Yet another normalization protocol is the calibration DNA gene set protocol in which each sample is normalized by the sum of calibration DNA genes. As used herein, calibration DNA genes
 30 are genes that produce reproducible expression values that are accurately measured. Such genes tend to have the same expression values on each of several different microarray s. The algorithm is the same as user normalization gene set protocol described above, but the set is predefined as the genes flagged as calibration DNA.

Yet another normalization protocol is the ratio median intensity correction protocol. This protocol is useful in embodiments in which a two-color fluorescence labeling and detection scheme is used. In the case where the two fluors in a two-color fluorescence labeling and detection scheme are Cy3 and Cy5, measurements are
 5 normalized by multiplying the ratio (Cy3/Cy5) by medianCy5/medianCy3 intensities. If background correction is enabled, measurements are normalized by multiplying the ratio (Cy3/Cy5) by (medianCy5-medianBkgdCy5) / (medianCy3-medianBkgdCy3) where medianBkgd means median background levels.

In some embodiments, intensity background correction is used to normalize
 10 measurements. The background intensity data from a spot quantification programs may be used to correct spot intensity. Background may be specified as either a global value or on a per-spot basis. If the array images have low background, then intensity background correction may not be necessary.

5.8 EXEMPLARY DISEASES

15 As discussed *supra*, the present invention provides methods for developing classifiers that can be used to determine whether a patient has a certain trait including a disease. Exemplary diseases that can be identified include asthma, cancers, common late-onset Alzheimer's disease, diabetes, heart disease, hereditary early-onset Alzheimer's disease (George-Hyslop *et al.*, 1990, *Nature* 347: 194), hereditary nonpolyposis colon
 20 cancer, hypertension, infection, maturity-onset diabetes of the young (Barbosa *et al.*, 1976, *Diabete Metab.* 2: 160), mellitus, nonalcoholic fatty liver (NAFL) (Younossi, *et al.*, 2002, *Hepatology* 35, 746-752), nonalcoholic steatohepatitis (NASH) (James & Day, 1998, *J. Hepatol.* 29: 495-501), non-insulin-dependent diabetes mellitus, and polycystic kidney disease (Reeders *et al.*, 1987, *Human Genetics* 76: 348).

25 Disease also includes, blood disorder, blood lipid disease, autoimmune disease, arthritis (including osteoarthritis, rheumatoid arthritis, lupus, allergies, juvenile rheumatoid arthritis and the like), bone or joint disorder, a cardiovascular disorder (including heart failure, congenital heart disease; rheumatic fever, valvular heart disease; cor pulmonale, cardiomyopathy, myocarditis, pericardial disease; vascular diseases such as
 30 atherosclerosis, acute myocardial infarction, ischemic heart disease and the like), obesity, respiratory disease (including asthma, pneumonitis, pneumonia, pulmonary infections, lung disease, bronchiectasis, tuberculosis, cystic fibrosis, interstitial lung disease, chronic bronchitis emphysema, pulmonary hypertension, pulmonary thromboembolism, acute respiratory distress syndrome and the like), hyperlipidemias, endocrine disorder, immune

disorder, infectious disease, muscle wasting and whole body wasting disorder, neurological disorders (including migraines, seizures, epilepsy, cerebrovascular diseases, alzheimers, dementia, parkinsons, ataxic disorders, motor neuron diseases, cranial nerve disorders, spinal cord disorders, meningitis and the like) including neurodegenerative and/or neuropsychiatric diseases and mood disorders (including schizophrenia, anxiety, bipolar disorder; manic depression and the like, skin disorder, kidney disease, scleroderma, stroke, hereditary hemorrhage telangiectasia, diabetes, disorders associated with diabetes (e.g., PVD), hypertension, Gaucher's disease, cystic fibrosis, sickle cell anemia, liver disease, pancreatic disease, eye, ear, nose and/or throat disease, diseases affecting the reproductive organs, gastrointestinal diseases (including diseases of the colon, diseases of the spleen, appendix, gall bladder, and others) and the like. For further discussion of human diseases, see Mendelian Inheritance in Man: A Catalog of Human Genes and Genetic Disorders by Victor A. McKusick (12th Edition (3 volume set) June 1998, Johns Hopkins University Press, ISBN: 0801857422) and Harrison's Principles of Internal Medicine by Braunwald, Fauci, Kasper, Hauser, Longo, & Jameson (15th Edition 2001), the entirety of which is incorporated herein.

Cancers that can be identified using the inventive techniques of the present invention include, but are not limited to, human sarcomas and carcinomas, e.g., fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, retinoblastoma; leukemias, e.g., acute lymphocytic leukemia and acute myelocytic leukemia (myeloblastic, promyelocyte, myelomonocytic, monocytic and erythroleukemia); chronic leukemia (chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia); and polycythemia vera, lymphoma (Hodgkin's disease

and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, and heavy chain disease.

5.9 EXEMPLARY DATABASE ARCHITECTURES

In some embodiments, training population 44, candidate molecular marker data structure 58, patient database 68, and/or classifier database 70 comprise or are stored in one or more data warehouses. Data warehouses are typically structured as either relational databases or multidimensional data cubes. This section describes relational databases and multidimensional data cube architectures that can be used to store training data, candidate molecular marker lists, patient molecular marker data and/or classifiers of the present invention. More information on relational databases and multidimensional data cubes is found in Berson and Smith, 1997, *Data Warehousing, Data Mining and OLAP*, McGraw-Hill, New York; Freeze, 2000, *Unlocking OLAP with Microsoft SQL Server and Excel 2000*, IDG Books Worldwide, Inc., Foster City, California; and Thomson, 1997, *OLAP Solutions: Building Multidimensional Information Systems*, Wiley Computer Publishing, New York.

5.9.1 DATA ORGANIZATION

Databases have typically been used for operational purposes, such as order entry, accounting and inventory control. More recently, corporations and scientific projects have been building databases, called data warehouses or large on-line analytical processing (OLAP) databases, explicitly for the purposes of exploration and analysis. The "data warehouse" can be described as a subject-oriented, integrated, time-variant, nonvolatile collection of data in support of management decisions. Data warehouses are built using both relational databases and specialized multidimensional structures called data cubes. In some embodiments a database stored in computer 10 or stored in a computer addressable by computer 10 across wide area network 34 is a relational database or a datacube.

5.9.2 RELATIONAL DATABASES

Relational databases organize data into tables where each row corresponds to a basic entity or fact and each column represents a property of that entity. For example, a table can represent transactions in a bank, where each row corresponds to a single transaction, and each transaction has multiple attributes, such as the transaction amount, the account balance, the bank branch, and the customer. The relational table is referred to as a relation, a row as a tuple, and a column as an attribute or field. The attributes within a relation can be partitioned into two types: dimensions and measures. Dimensions and

measures are similar to independent and dependent variables in traditional analysis. For example, the bank branch and the customer would be dimensions, while the account balance would be a measure. A single relational database will often describe many heterogeneous but interrelated entities. For example, a database designed for a restaurant chain might maintain information about employees, products, and sales. The database schema defines the relations in a database, the relationships between those relations, and how the relations classify the entities of interest.

5.9.3 DATA CUBES

A data warehouse can be constructed as a relational database using either a star or snowflake schema and will provide a conceptual classifier of a multidimensional data set. Each axis in the corresponding data cube represents a dimension in a relational schema and consists of every possible value for that dimension. For example, an axis corresponding to states would have fifty values, one for each state. Each cell in the data cube corresponds to a unique combination of values for the dimensions. For instance, if there are two dimensions, "state" and "product", then there would be a cell for every unique combination of the two, e.g., one cell each for (California, Tea), (California, Coffee), (Florida, Tea), (Florida, Coffee), etc. Each cell contains one value per measure of the data cube. So if product production and consumption information is needed, then each cell would contain two values, one for the number of products of each type consumed in that state, and one for the number of products of each type produced in that state. Dimensions within a data warehouse are often augmented with a hierarchical structure. If each dimension has a hierarchical structure, then the data warehouse is not a single data cube but rather a lattice of data cubes.

5.10 EXEMPLARY PATIENT DATABASE

This section provides a more detailed description of a patient database 68 in accordance with one aspect of the invention. As described in Section 5.1, an exemplary patient database 68 includes a plurality of patient records 500 (Fig. 6). There is no limit on the number of patient records 500 that can be held in patient database 68. Database 68 can hold as few as one patient record 500. More typically, database 68 holds between 1 and 100 patient records, more than 100 patient records, more than a thousand patient records, more than ten thousand patient records, more than 100 thousand patient records, between 1 patient record and one million patient records, or more. Each patient record 500 preferably includes a patient identifier 502. As those skilled in database arts will appreciate, a patient

identifier 502 need not be explicitly enumerated in certain database systems. For instance, in some systems, a patient identifier 502 can simply be a patient record 500 identifier. However, in some embodiments, a patient identifier 502 can be a number that uniquely identifies a patient within a health care program or clinical trial.

5 An advantage of database 68 is that it has the capability of tracking molecular marker data profile 504 and trait characterization information 510 for each patient registered in database 68. In some embodiments, a molecular profile 504 is the abundance levels of a plurality of molecular marker products in blood specimens obtained from a patient in accordance with Section 5.2. In some embodiments, such abundance levels are
10 normalized using any of the techniques disclosed in Section 5.7.

 In some embodiments, a molecular profile 504 comprises the processed microarray image data from the biological specimen obtained from the patient. In one example, molecular profile data 504 comprises molecular marker abundance information for all or a portion of the cellular constituents represented in a microarray, optional background signal
15 information, and optional associated annotation information describing the probes used for the respective molecular marker. Molecular markers include, but are not limited to RNA (e.g., mRNA) and protein.

 In some embodiments, a molecular profile 504 represents the transcriptional state of cellular constituents in a biological specimen. However, in other embodiments, a
20 molecular profile 504 can track aspects of the biological state other than or in addition to transcriptional state. Such other aspects of the biological state include, but are not limited to, the translational state, the activity state of cellular constituents in a biological sample. In some embodiments, for example, molecular profile 504 data is, in fact, protein levels for various proteins in the blood taken from the patient. Thus, in some embodiments,
25 molecular profiles 504 comprise amounts or concentrations of the molecular markers in biological specimens obtained in accordance with Section 5.2.

 In one embodiment, the amount of at least one molecular marker that is tracked in a molecular profile 504 comprises abundances of at least one RNA species present in one or more cells in the blood obtained from the patient. Such abundances can be measured by a
30 method comprising contacting a gene transcript array with RNA derived from one or more cells of the biological specimen, or with cDNA derived therefrom. A gene transcript array comprises a surface with attached nucleic acids or nucleic acid mimics. The nucleic acids or nucleic acid mimics are capable of hybridizing with the RNA species or with cDNA derived from the RNA species. In one particular embodiment, the abundance of the RNA

is measured by contacting a gene transcript array with the RNA from one or more cells of the biological specimen, or with nucleic acid derived from the RNA, such that the gene transcript array comprises a positionally addressable surface with attached nucleic acids or nucleic acid mimics, where the nucleic acids or nucleic acid mimics are capable of
5 hybridizing with the RNA species, or with nucleic acid derived from the RNA species.

In some embodiments, a molecular profile 504 can include abundance information or activity information about ten or more molecular markers (e.g., genes or proteins), between ten and one thousand molecular markers, between one thousand and twenty thousand molecular markers, or more than twenty thousand molecular markers.

10 In some embodiments, in addition to or rather than providing abundance information or activity information for molecular markers, a molecular profile 504 tracks polymorphism information. Such polymorphism information includes, but is not limited to, single nucleotide polymorphisms (SNPs), SNP haplotypes, microsatellite markers, restriction fragment length polymorphisms (RFLPs), short tandem repeats, sequence length
15 polymorphisms, DNA methylation, random amplified polymorphic DNA (RAPD), amplified fragment length polymorphisms (AFLP), and "simple sequence repeats." For more information on such polymorphisms, see generally, *The DNA Revolution* by Andrew H. Paterson 1996 (Chapter 2) in: *Genome Mapping in Plants* (ed. Andrew H. Paterson) by Academic Press/R. G. Landis Company, Austin, Tex., 7-21, which is hereby incorporated
20 herein by reference in its entirety

SNPs occur approximately once every 600 base pairs in the genome. See, for example, Kruglyak and Nickerson, 2001, *Nature Genetics* 27, 235. Alleles making up blocks of such SNPs in close physical proximity are often correlated, resulting in reduced genetic variability and defining a limited number of "SNP haplotypes" each of which
25 reflects descent from a single ancient ancestral chromosome. See Fullerton *et al.*, 2000, *Am. J. Hum. Genet.* 67, 881. Such haplotype structure is used in some embodiments of the present invention. Patil *et al.* found that a very dense set of SNPs is required to capture all the common haplotype information. See Patil *et al.*, 2001, *Science* 294, 1719-1723. DNA methylation is described in Grunau *et al.*, 2003, *Nucleic Acids Res.* 31, pp. 75-77.

30 RFLPs are the product of allelic differences between DNA restriction fragments caused by nucleotide sequence variability. As is well known to those of skill in the art, RFLPs are typically detected by extraction of genomic DNA and digestion with a restriction endonuclease. Generally, the resulting fragments are separated according to size and hybridized with a probe; single copy probes are preferred. As a result, restriction

fragments from homologous chromosomes are revealed. Differences in fragment size among alleles represent an RFLP (see, for example, Helentjaris *et al*, 1985, Plant Mol Bio. 5:109-118, and U.S. Pat. No. 5,324,631).

The phrase "random amplified polymorphic DNA" or "RAPD" refers to the
5 amplification product of the distance between DNA sequences homologous to a single oligonucleotide primer appearing on different sites on opposite strands of DNA. Mutations or rearrangements at or between binding sites will result in polymorphisms as detected by the presence or absence of amplification product (see, for example, Welsh and McClelland, 1990, Nucleic Acids Res. 18:7213-7218; Hu and Quiros, 1991, Plant Cell
10 Rep. 10:505-511). AFLP technology refers to a process that is designed to generate large numbers of randomly distributed molecular markers (see, for example, European Patent Application No. 0534858 A1).

"Simple sequence repeats" or "SSRs" are di-, tri- or tetra-nucleotide tandem repeats within a genome. The repeat region can vary in length between genotypes while
15 the DNA flanking the repeat is conserved such that the same primers will work in a plurality of genotypes. A polymorphism between two genotypes represents repeats of different lengths between the two flanking conserved DNA sequences (see, for example, Akagi *et al*, 1996, Theor. Appl. Genet. 93, 1071-1077; Bligh *et al*, 1995, Euphytica 86:83-85; Struss *et al*, 1998, Theor. Appl. Genet. 97, 308-315; Wu *et al*, 1993, Mol Gen.
20 Genet. 241, 225-235; and U.S. Pat. No. 5,075,217). SSR are also known as satellites or microsatellites.

In addition to molecular profiles 50, patient records 500 include trait characterizations 510. In some embodiments, a trait characterization 510 comprises observations made by a patient's physician. In some instances, the observations made by a
25 physician include a code from the International Classification of Diseases, 9th Revision, prepared by the Department of Health and Human Services (ICD-9 codes), or an equivalent, and dates such observations were made.

5.11 EXEMPLARY GENES AS CANDIDATE MOLECULAR MARKERS

Non-limiting examples of genes useful as molecular markers for use in the
30 invention can include, but are not limited to, genes specific for or involved in a particular biological process, such as apoptosis, differentiation, stress response, aging, proliferation, etc.; cellular mechanism genes, e.g., cell-cycle, signal transduction, metabolism of toxic compounds, and the like; disease associated genes, e.g., genes involved in cancer, schizophrenia, diabetes, high blood pressure, atherosclerosis, viral-host interaction and

infection and the like. Exemplary genes can also include immune responsive genes.

Further examples of genes can include, but are not limited to, oncogenes whose expression within a cell induces that cell to become converted from a normal cell into a tumor cell.

See for example Hanahan & Weinberg, 2000, *Cell* 100:57; Yokota., 2000, *Carcinogenesis*

5 21:497. Further examples of genes can include, but are not limited to cytokine genes. See, for example, Rubinstein *et al*, 1998, *Cytokine Growth Factor Rev.* 9:175-81. Other examples of genes can include idiotype protein genes (*e.g.*, Benezra., *et al*, 2001 *Oncogene* 20:8334-41; Norton, 2000, *J. Cell Sci.* 113:3897), prion genes (*e.g.*, Prusiner *et al*, 1998, *Cell* 93:337-48; Safar & Prusiner, 1998, *Prog. Brain Res.* 117:421); genes that
10 express molecules that induce angiogenesis (*e.g.*, Gould & Wagner, 2002, *Hum. Pathol.* 33:1061); genes encoding adhesion molecules (*e.g.*, Chothia, & Jones, 1997, *Annu. Rev. Biochem.* 66:823; Parise *et al*, 2000, *Semin. Cancer Biol.* 10:407-14); genes encoding cell surface receptors (*e.g.*, Deller and Jones, 2000, *Curr. Opin. Struct. Biol.* 10:213); genes of proteins that are involved in metastasizing and/or invasive processes (*e.g.*, Boyd, 1996,
15 *Cancer Metastasis Rev.* 15:77; Yokota, 2000, *Carcinogenesis* 21:497); genes of proteases as well as of molecules that regulate apoptosis and the cell cycle (*e.g.*, Matrisian, 1999, *Curr. Biol.* 9:R776; Krepela, 2001, *Neoplasma* 48:332; Basbaum and Werb, 1996, *Curr. Opin. Cell Biol.* 8:731; Birkedal-Hansen *et al*, 1993, *Crit. Rev. Oral Biol. Med.* 4:197-250; Mignatti and Rifkin, 1993, *Physiol. Rev.* 73:161; Stetler-Stevenson *et al.*, 1993,
20 *Annu. Rev. Cell Biol.* 9:541; Brinkerhoff and Matrisan, 2002, *Nature Reviews* 3:207; Strasser. *et al*, 2000, *Annu. Rev. Biochem.* 69:217; Chao and Korsmeyer, 1998, *Annu. Rev. Immunol.* 16:395; Mullauer *et al*, 2001, *Mutat. Res.* 488:21 1; Fotadar *et al*, 1996, *Prog. Cell Cycle Res.* 2:147; Reed., 2000, *Am. J. Pathol.* 157:1415; D'Ari, 2001, *Bioassays* 23:563); or multi-drug resistance genes, such as the MDRI gene. In one embodiment, a
25 gene is an immune response gene or a non-immune response gene such as cytokines (*e.g.*, interleukins and interferons such as TNF-alpha, IL-10, IL-12, IL-2, IL-4, IL-10, IL-12, IL-13, TGF-Beta, IFN-gamma; immunoglobulins, complement and the like). See, for example, Bellardelli, 1995, *Role of interferons and other cytokines in the regulation of the immune response* *APMIS* 103: 161.

30

5.12 CLUSTERING TECHNIQUES

In some embodiments, clustering is used. For instance, clustering can be used in step 204 to visualize the relationship between the data measured for a plurality of molecular markers in step 202. In some embodiments, any of the clustering techniques described in Draghici, *Data Analysis Tools For DNA Microarrays*, 2003, Chapman &

Hall, CRC Press, New York, pp. 263-297, which is hereby incorporated by reference in its entirety, are used in the present invention. Clustering is also described on pages 211-256 of Duda and Hart, *Pattern Classification and Scene Analysis*, 1973, John Wiley & Sons, Inc., New York, which is hereby incorporated by reference. As described in Section 6.7 of

5 Duda, the clustering problem is described as one of finding natural groupings in a dataset. To identify natural groupings, two issues are addressed. First, a way to measure similarity (or dissimilarity) between two samples is determined. This metric (similarity measure) is used to ensure that the samples in one cluster are more like one another than they are to samples in other clusters. Second, a mechanism for partitioning the data into clusters

10 using the similarity measure is determined.

Similarity measures are discussed in Section 6.7 of Duda, where it is stated that one way to begin a clustering investigation is to define a distance function and to compute the matrix of distances between all pairs of samples in a dataset. If distance is a good measure of similarity, then the distance between samples in the same cluster will be significantly

15 less than the distance between samples in different clusters. However, as stated on page 215 of Duda, clustering does not require the use of a distance metric. For example, a nonmetric similarity function $s(x, x')$ can be used to compare two vectors x and x' . Conventionally, $s(x, x')$ is a symmetric function whose value is large when x and x' are somehow "similar". An example of a nonmetric similarity function $s(x, x')$ is provided on

20 page 216 of Duda.

Once a method for measuring "similarity" or "disimilarity" between points in a dataset has been selected, clustering requires a criterion function that measures the clustering quality of any partition of the data. Partitions of the data set that extremize the criterion function are used to cluster the data. See page 217 of Duda. Criterion functions

25 are discussed in Section 6.8 of Duda.

More recently, Duda et al., *Pattern Classification*, 2nd edition, John Wiley & Sons, Inc. New York, has been published. Pages 537-563 describe clustering in detail. More information on clustering techniques can be found in Kaufman and Rousseeuw, 1990, *Finding Groups in Data: An Introduction to Cluster Analysis*, Wiley, New York, NY;

30 Everitt, 1993, *Cluster analysis* (3d ed.), Wiley, New York, NY; and Backer, 1995, *Computer-Assisted Reasoning in Cluster Analysis*, Prentice Hall, Upper Saddle River, New Jersey. Now that an overview of clustering techniques has been given, more specific examples of clustering that can be performed in the methods described in Section 5.1 is presented.

5.12.1 HIERARCHICAL CLUSTERING TECHNIQUES

Hierarchical cluster analysis is a statistical method for finding relatively homogenous clusters of elements based on measured data. Consider a sequence of partitions of n samples into c clusters. The first of these is a partition into n clusters, each cluster containing exactly one sample. The next is a partition into $n-1$ clusters, the next is partition into $n-2$, and so on until the n^{th} , in which all the samples form one cluster. Level k in the sequence of partitions occurs when $c = n - k + 1$. Thus, level one corresponds to n clusters and level n corresponds to one cluster. Given any two samples x and x^* , at some level they will be grouped together in the same cluster. If the sequence has the property that whenever two samples are in the same cluster at level k they remain together at all higher levels, then the sequence is said to be a hierarchical clustering. Duda et al., 2001, Pattern Classification, 2nd edition, John Wiley & Sons, New York, 2001: 551. Examples of hierarchical clustering includes agglomerative clustering using nearest-neighbor algorithm, farthest-neighbor algorithm, the average linkage algorithm, the centroid algorithm, or the sum-of-squares algorithm. See, for example WO03 100557.

5.12.1.1 CLUSTERING WITH PEARSON CORRELATION COEFFICIENTS

In some embodiments of the present invention, molecular marker data data is clustered using agglomerative hierarchical clustering with Pearson correlation coefficients. In this form of clustering, similarity is determined using Pearson correlation coefficients between sets of molecular marker data measurements. Other metrics that can be used, in addition to the Pearson correlation coefficient, include but are not limited to, a Euclidean distance, a squared Euclidean distance, a Euclidean sum of squares, a Manhattan metric, and a squared Pearson correlation coefficient. Such metrics can be computed using SAS (Statistics Analysis Systems Institute, Gary, North Carolina) or S-Plus (Statistical Sciences, Inc., Seattle, Washington).

5.12.1.2 DIVISIVE CLUSTERING

In some embodiments, the hierarchical clustering technique used to cluster molecular marker data measurements is a divisive clustering procedure. Divisive (top-down clustering) procedures start with all of the samples in one cluster and form the sequence by successfully splitting clusters. Divisive clustering techniques are classified as either a polymeric or a monothetic method. A polythetic approach divides clusters into arbitrary subsets.

5.12.2 K-MEANS CLUSTERING

In k-means clustering, sets of molecular marker data measurements are randomly assigned to K user specified clusters. The centroid of each cluster is computed by averaging the value of the vectors in each cluster. Then, for each $i = 1, \dots, N$, the distance between vector x_i and each of the cluster centroids is computed. Each vector x_i is then reassigned to the cluster with the closest centroid. Next, the centroid of each affected cluster is recalculated. The process iterates until no more reassignments are made. See, for example, Duda *et al*, 2001, *Pattern Classification*, John Wiley & Sons, New York, NY, pp. 526-528. A related approach is the fuzzy k-means clustering algorithm, which is also known as the fuzzy c-means algorithm, hi the fuzzy k-means clustering algorithm, the assumption that every set of molecular marker data measurements is in exactly one cluster at any given time is relaxed so that every set has some graded or "fuzzy" membership in a cluster. See Duda *et al*, 2001, *Pattern Classification*, John Wiley & Sons, New York, NY, pp. 528-530.

5.12.3 JARVIS-PATRICK CLUSTERING

Jarvis-Patrick clustering is a nearest-neighbor non-hierarchical clustering method in which a set of objects is partitioned into clusters on the basis of the number of shared nearest-neighbors. In the standard implementation advocated by Jarvis and Patrick, 1973, *IEEE Trans. Comput*, C-22: 1025-1034, a preprocessing stage identifies the K nearest-neighbors of each object in the dataset. In the subsequent clustering stage, two objects i and j join the same cluster if (i) i is one of the K nearest-neighbors of j , (ii) j is one of the K nearest-neighbors of i , and (iii) i and j have at least k_{mn} of their K nearest-neighbors in common, where K and k_{mn} are user-defined parameters. The method has been widely applied to clustering chemical structures on the basis of fragment descriptors and has the advantage of being much less computationally demanding than hierarchical methods, and thus more suitable for large databases. Jarvis-Patrick clustering can be performed using the Jarvis-Patrick Clustering Package 3.0 (Barnard Chemical Information, Ltd., Sheffield, United Kingdom).

5.13 MOLECULAR MARKERS

Molecular marker is used herein to mean a gene or genetic element. All genes and genetic elements are considered molecular markers, but the invention teaches how to identify molecular markers useful for diagnosing a trait of interest.

5.14 REPRESENTATIVE MATHEMATICAL MODELS THAT CAN BE USED TO BUILD CLASSIFIERS

This section describes various mathematical models that can be used to build classifier in accordance with the methods of the present invention.

5

5.14.1 REGRESSION CLASSIFIERS

In some embodiments, the classifier constructed in step 216 is a regression classifier, preferably a logistic regression classifier. Such a regression classifier includes a coefficient for each of the molecular markers selected in the last instance of step 214. In such embodiments, the coefficients for the regression classifier are computed using, for example, a maximum likelihood approach. In such a computation, the data measured for the molecular markers in step 206 (*e.g.*, RT-PCR data) is used. In particular embodiments, molecular marker data from only two trait subgroups is used and the dependent variable is absence or presence of a particular trait in the subjects for which molecular marker data is available. As in the case of step 210, the two different trait subgroups can, for example, respectively represent a diseased and nondiseased state, a first diseased state (*e.g.* liver cancer) and a second phenotypically similar (*e.g.* hepatitis B) or unrelated diseased state (*e.g.*, Alzheimer's disease), those subjects that are responsive to drug therapy and those subjects that are not responsive to drug therapy, or subjects that have been subjected to a perturbation (*e.g.*, drug treatment) versus those subjects that have not been subjected to a perturbation.

In another specific embodiment, training population 44 consists of a plurality of trait subgroups (*e.g.*, three or more trait subgroups, four or more specific trait subgroups, *etc.*). In this specific embodiment, a generalization of the logistic regression model that handles multicategory responses can be used in step 216 to develop a classifier that discriminates between the various trait subgroups found in the training population. For example, measured data for selected molecular markers can be applied to any of the multicategory logit models described in Agresti, *An Introduction to Categorical Data Analysis*, 1996, John Wiley & Sons, Inc., New York, Chapter 8, which is hereby incorporated herein by reference in its entirety, in order to develop a classifier capable of discriminating between any of a plurality of trait subgroups represented in a training population.

5.14.2 NEURAL NETWORKS

The present invention is not limited to the use of logistic regression. In some embodiments, the data measured for the molecular markers in step 206 (*e.g.*, RT-PCR data) can be used to train a neural network.

5 In some embodiments, a neural network is derived in each successive instance of step 216 of Fig. 2A using the combination of molecular markers selected in the corresponding instance of step 214 of Fig. 2A. A neural network is a two-stage regression or classification classifier. A neural network has a layered structure that includes a layer of input units (and the bias) connected by a layer of weights to a layer of output units. For
10 regression, the layer of output units typically includes just one output unit. However, neural networks can handle multiple quantitative responses in a seamless fashion.

 In multilayer neural networks, there are input units (input layer), hidden units (hidden layer), and output units (output layer). There is, furthermore, a single bias unit that is connected to each unit other than the input units. Neural networks are described in
15 Duda *et al*, 2001, *Pattern Classification*, Second Edition, John Wiley & Sons, Inc., New York; and Hastie *et al*, 2001, *The Elements of Statistical Learning*, Springer-Verlag, New York.

 The basic approach to the use of neural networks is to start with an untrained network, present a training pattern to the input layer, and to pass signals through the net
20 and determine the output at the output layer. These outputs are then compared to the target values; any difference corresponds to an error. This error or criterion function is some scalar function of the weights and is minimized when the network outputs match the desired outputs. Thus, the weights are adjusted to reduce this measure of error. For regression, this error can be sum-of-squared errors. For classification, this error can be
25 either squared error or cross-entropy (deviation). See, *e.g.*, Hastie *et al*, 2001, *The Elements of Statistical Learning*, Springer-Verlag, New York.

 Three commonly used training protocols are stochastic, batch, and on-line. In stochastic training, patterns are chosen randomly from the training set and the network weights are updated for each pattern presentation. Multilayer nonlinear networks trained
30 by gradient descent methods such as stochastic back-propagation perform a maximum-likelihood estimation of the weight values in the classifier defined by the network topology. In batch training, all patterns are presented to the network before learning takes place. Typically, in batch training, several passes are made through the training data. In online training, each pattern is presented once and only once to the net.

In some embodiments, consideration is given to starting values for weights. If the weights are near zero, then the operative part of the sigmoid commonly used in the hidden layer of a neural network (see, *e.g.*, Hastie *et al*, 2001, *The Elements of Statistical Learning*, Springer-Verlag, New York) is roughly linear, and hence the neural network
5 collapses into an approximately linear classifier. In some embodiments, starting values for weights are chosen to be random values near zero. Hence the classifier starts out nearly linear, and becomes nonlinear as the weights increase. Individual units localize to directions and introduce nonlinearities where needed. Use of exact zero weights leads to zero derivatives and perfect symmetry, and the algorithm never moves. Alternatively,
10 starting with large weights often leads to poor solutions.

Since the scaling of inputs determines the effective scaling of weights in the bottom layer, it can have a large effect on the quality of the final solution. Thus, in some embodiments, at the outset all expression values are standardized to have mean zero and a standard deviation of one. This ensures all inputs are treated equally in the regularization
15 process, and allows one to choose a meaningful range for the random starting weights. With standardization inputs, it is typical to take random uniform weights over the range $[-0.7, +0.7]$.

A recurrent problem in the use of three-layer networks is the optimal number of hidden units to use in the network. The number of inputs and outputs of a three-layer
20 network are determined by the problem to be solved. In embodiments of the present invention, the number of inputs for a given neural network can, in some embodiments, equal the number of molecular markers selected in the corresponding instance of step 214. In other embodiments, for each input, two or more molecular markers will be selected (for example wherein ratios of genes (A/B) are utilized. The number of outputs for the neural
25 network will typically be just one (ie wherein the output neuron is one dimensional *e.g.* health vs. disease). If there are additional input dimensions, new additional output neurons may be created. In some embodiments more than one output is used so that more than just two states can be defined by the network. If too many hidden units are used in a neural network, the network will have too many degrees of freedom and is trained too long, there
30 is a danger that the network will overfit the data. If there are too few hidden units, the training set cannot be learned. Generally speaking, however, it is better to have too many hidden units than too few. With too few hidden units, the classifier might not have enough flexibility to capture the nonlinearities in the data; with too many hidden units, the extra weight can be shrunk towards zero if appropriate regularization or pruning, as described

below, is used. In typical embodiments, the number of hidden units in somewhere in the range of 5 to 100, with the number increasing with the number of inputs and number of training cases.

One general approach to determining the number of hidden units to use is to apply a regularization approach. La the regularization approach, a new criterion function is constructed that depends not only on the classical training error, but also on classifier complexity. Specifically, the new criterion function penalizes highly complex classifiers; searching for the minimum in this criterion is to balance error on the training set with error on the training set plus a regularization term, which expresses constraints or desirable properties of solutions:

$$\tilde{J} = J_{\text{pat}} + \lambda J_{\text{reg.}}$$

The parameter λ is adjusted to impose the regularization more or less strongly. In other words, larger values for λ will tend to shrink weights towards zero: typically cross-validation with a validation set is used to estimate λ . This validation set can be obtained by setting aside a random subset of the population measured in step 202 of Fig. 2A. Other forms of penalty have been proposed, for example the weight elimination penalty (see, e.g., Hastie *et al*, 2001, *The Elements of Statistical Learning*, Springer-Verlag, New York).

Another approach to determine the number of hidden units to use is to eliminate - prune - weights that are least needed. In one approach, the weights with the smallest magnitude are eliminated (set to zero). Such magnitude-based pruning can work, but is nonoptimal; sometimes weights with small magnitudes are important for learning and training data. In some embodiments, rather than using a magnitude-based pruning approach, WaId statistics are computed. The fundamental idea in WaId Statistics is that they can be used to estimate the importance of a hidden unit (weight) in a classifier. Then, hidden units having the least importance are eliminated (by setting their input and output weights to zero). Two algorithms in this regard are the *Optimal Brain Damage* (OBD) and the *Optimal Brain Surgeon* (OBS) algorithms that use second-order approximation to predict how the training error depends upon a weight, and eliminate the weight that leads to the smallest increase in training error.

Optimal Brain Damage and Optimal Brain Surgeon share the same basic approach of training a network to local minimum error at weight w , and then pruning a weight that leads to the smallest increase in the training error. The predicted functional increase in the error for a change in full weight vector δw is:

$$\delta J = \left(\frac{\partial J}{\partial w} \right)' \cdot \delta w + \frac{1}{2} \delta w' \cdot \frac{\partial^2 J}{\partial w^2} \cdot \delta w + O(\|\delta w\|^3)$$

where $\frac{\partial^2 J}{\partial w^2}$ is the Hessian matrix. The first term vanishes because we are at a local minimum in error; third and higher order terms are ignored. The general solution for minimizing this function given the constraint of deleting one weight is:

$$\delta w = - \frac{w_q}{[\mathbf{H}^{-1}]_{qq}} \mathbf{H}^{-1} \cdot \mathbf{u}_q \quad \text{and} \quad L_q = \frac{1}{2} - \frac{w_q^2}{[\mathbf{H}^{-1}]_{qq}}$$

Here, \mathbf{u}_q is the unit vector along the q th direction in weight space and L_q is approximation to the saliency of the weight q - the increase in training error if weight q is pruned and the other weights updated δw . These equations require the inverse of \mathbf{H} . One method to calculate this inverse matrix is to start with a small value, $\mathbf{H}_0^{-1} = \alpha^{-1} \mathbf{I}$, where α is a small parameter - effectively a weight constant. Next the matrix is updated with each pattern according to

$$\mathbf{H}_{m+1}^{-1} = \mathbf{H}_m^{-1} - \frac{\mathbf{H}_m^{-1} \mathbf{X}_{m+1} \mathbf{X}_{m+1}^T \mathbf{H}_m^{-1}}{\frac{n}{a_m} + \mathbf{X}_{m+1}^T \mathbf{H}_m^{-1} \mathbf{X}_{m+1}} \quad \text{Eqn. 1}$$

where the subscripts correspond to the pattern being presented and a_m decreases with m . After the full training set has been presented, the inverse Hessian matrix is given by $\mathbf{H}^{-1} = \mathbf{H}_n^{-1}$. In algorithmic form, the Optimal Brain Surgeon method is:

' begin initialize Π_H, W, θ

train a reasonably large network to minimum error

do compute \mathbf{H}^{-1} by Eqn. 1

$$q \leftarrow \arg \min_q w_q^2 / (2[\mathbf{H}^{-1}]_{qq}) \quad (\text{saliency } L_q)$$

20

$$w \leftarrow w - \frac{w_q}{[\mathbf{H}^{-1}]_{qq}} \mathbf{H}^{-1} \mathbf{e}_q \quad (\text{saliency } L_q)$$

until $J(w) > \theta$

return w

end

The Optimal Brain Damage method is computationally simpler because the calculation of the inverse Hessian matrix in line 3 is particularly simple for a diagonal matrix. The above algorithm terminates when the error is greater than a criterion initialized to be θ . Another approach is to change line 6 to terminate when the change in $J(w)$ due to elimination of a weight is greater than some criterion value.

In some embodiments, the back-propagation neural network (see, for example Abdi, 1994, "A neural network primer", J. Biol System. 2, 247-283) containing a single hidden layer often neurons (ten hidden units) found in EasyNN-Plus version 4.0g software package (Neural Planner Software Inc.) is used. In one specific example, parameter values within the EasyNN-Plus program were set as follows: learning parameter = 0.6, and momentum parameter = 0.8. In some embodiments in which the EasyNN-Plus version 4.0g software package is used, "outlier" samples are identified by performing twenty independently-seeded trials involving 20,000 learning cycles each.

5.14.3 CLUSTERING

In some embodiments, the expression values for select genes are used to cluster a training set. For example, consider the case in which ten genes are used. Each member m of the training population will have expression values for each of the ten genes. Such values from a member m in the training population define the vector:

X_{1m}	X_{2m}	X_{3m}	X_{4m}	X_{5m}	X_{6m}	X_{7m}	X_{8m}	X_{9m}	X_{10m}
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where X_{jm} is the expression level of the j^{th} gene in organism m . If there are m organisms in the training set, selection of i genes will define m vectors. Note that the methods of the present invention do not require that each expression value of every single gene used in the vectors be represented in every single vector m . In other words, data from a subject in which one of the i^{th} genes is not found can still be used for clustering. In such instances, the missing expression value is assigned either a "zero" or some other normalized value. In some embodiments, prior to clustering, the gene expression values are normalized to have a mean value of zero and unit variance.

Those members of the training population that exhibit similar expression patterns across the training group will tend to cluster together. A particular combination of genes of the present invention is considered to be a good classifier in this aspect of the invention when the vectors cluster into the trait groups found in the training population. For instance, if the training population includes patients with no osteoarthritis, mild

osteoarthritis, moderate osteoarthritis, marked osteoarthritis, and severe osteoarthritis an ideal clustering classifier will cluster the population into five groups, with each group uniquely representing either absence or one of the four stages of osteoarthritis. In some embodiments, the clustering classifier simply clusters the population into a first subgroup
5 (a first cluster) that does not have osteoarthritis and a second subgroup (a second cluster) that has osteoarthritis. In some embodiments, the classifier clusters the data into a first subgroup that has a particular stage of osteoarthritis (e.g., mild) and two or more subgroups that do not include subjects having the particular stage of osteoarthritis represented in the first subgroup.

10 Clustering is described on pages 211-256 of Duda and Hart, *Pattern Classification and Scene Analysis*, 1973, John Wiley & Sons, Inc., New York. As described in Section 6.7 of Duda, the clustering problem is described as one of finding natural groupings in a dataset. To identify natural groupings, two issues are addressed. First, a way to measure similarity (or dissimilarity) between two samples is determined. This metric (similarity
15 measure) is used to ensure that the samples in one cluster are more like one another than they are to samples in other clusters. Second, a mechanism for partitioning the data into clusters using the similarity measure is determined.

Similarity measures are discussed in Section 6.7 of Duda, where it is stated that one way to begin a clustering investigation is to define a distance function and to compute the
20 matrix of distances between all pairs of samples in a dataset. If distance is a good measure of similarity, then the distance between samples in the same cluster will be significantly less than the distance between samples in different clusters. However, as stated on page 215 of Duda, clustering does not require the use of a distance metric. For example, a nonmetric similarity function $s(x, x')$ can be used to compare two vectors x and x' .
25 Conventionally, $s(x, x')$ is a symmetric function whose value is large when x and x' are somehow "similar". An example of a nonmetric similarity function $s(x, x')$ is provided on page 216 of Duda.

Once a method for measuring "similarity" or "dissimilarity" between points in a dataset has been selected, clustering requires a criterion function that measures the
30 clustering quality of any partition of the data. Partitions of the data set that extremize the criterion function are used to cluster the data. See page 217 of Duda. Criterion functions are discussed in Section 6.8 of Duda.

More recently, Duda *et al*, *Pattern Classification*, 2nd edition, John Wiley & Sons, Inc. New York, has been published. Pages 537-563 describe clustering in detail. More

information on clustering techniques can be found in Kaufman and Rousseeuw, 1990, *Finding Groups in Data: An Introduction to Cluster Analysis*, Wiley, New York, NY; Everitt, 1993, *Cluster analysis* (3d ed.), Wiley, New York, NY; and Backer, 1995, *Computer-Assisted Reasoning in Cluster Analysis*, Prentice Hall, Upper Saddle River, New Jersey. Particular exemplary clustering techniques that can be used in the present invention include, but are not limited to, hierarchical clustering (agglomerative clustering using nearest-neighbor algorithm, farthest-neighbor algorithm, the average linkage algorithm, the centroid algorithm, or the sum-of-squares algorithm), k-means clustering, fuzzy k-means clustering algorithm, and Jarvis-Patrick clustering.

5.14.4 PRINCIPAL COMPONENT ANALYSIS

Principal component analysis (PCA) has been proposed to analyze gene expression data. Principal component analysis is a classical technique to reduce the dimensionality of a data set by transforming the data to a new set of variable (principal components) that summarize the features of the data. See, for example, Jolliffe, 1986, *Principal Component Analysis*, Springer, New York. Principal components (PCs) are uncorrelated and are ordered such that the k^{th} PC has the M_i largest variance among PCs. The k^{th} PC can be interpreted as the direction that maximizes the variation of the projections of the data points such that it is orthogonal to the first $k - 1$ PCs. The first few PCs capture most of the variation in the data set. In contrast, the last few PCs are often assumed to capture only the residual 'noise' in the data.

PCA can also be used to create a classifier in accordance with the present invention. In such an approach, vectors for the select genes described in the present invention can be constructed in the same manner described for clustering above. In fact, the set of vectors, where each vector represents the expression values for the select genes from a particular member of the training population, can be considered a matrix. In some embodiments, this matrix is represented in a Free-Wilson method of qualitative binary description of monomers (Kubinyi, 1990, *3D QSAR in drug design theory methods and applications*, Pergamon Press, Oxford, pp 589-638), and distributed in a maximally compressed space using PCA so that the first principal component (PC) captures the largest amount of variance information possible, the second principal component (PC) captures the second largest amount of all variance information, and so forth until all variance information in the matrix has been accounted for.

Then, each of the vectors (where each vector represents a member of the training population) is plotted. Many different types of plots are possible. In some embodiments, a

one-dimensional plot is made. In this one-dimensional plot, the value for the first principal component from each of the members of the training population is plotted. In this form of plot, the expectation is that members of a first subgroup (e.g. those subjects that do not have osteoarthritis) will cluster in one range of first principal component values and

5 members of a second subgroup (e.g., those subjects that have osteoarthritis) will cluster in a second range of first principal component values.

In one ideal example, the training population comprises two subgroups: "control" and "patients with osteoarthritis." The first principal component is computed using the molecular marker expression values for the select genes of the present invention across the

10 entire training population data set. Then, each member of the training set is plotted as a function of the value for the first principal component. In this ideal example, those members of the training population in which the first principal component is positive are the "responders" and those members of the training population in which the first principal component is negative are "patients with osteoarthritis."

15 In some embodiments, the members of the training population are plotted against more than one principal component. For example, in some embodiments, the members of the training population are plotted on a two-dimensional plot in which the first dimension is the first principal component and the second dimension is the second principal component. In such a two-dimensional plot, the expectation is that members of each

20 subgroup represented in the training population will cluster into discrete groups. For example, a first cluster of members in the two-dimensional plot will represent subjects with mild osteoarthritis, a second cluster of members in the two-dimensional plot will represent subjects with moderate osteoarthritis, and so forth.

In some embodiments, the members of the training population are plotted against

25 more than two principal components and a determination is made as to whether the members of the training population are clustering into groups that each uniquely represents a subgroup found in the training population. In some embodiments, principal component analysis is performed by using the *R mva* package (Anderson, 1973, *Cluster Analysis for applications*, Academic Press, New York 1973; Gordon, *Classification*, Second Edition, Chapman and Hall, CRC, 1999.). Principal component analysis is further described in

30 Duda, *Pattern Classification*, Second Edition, 2001, John Wiley & Sons, Inc.

5.14.5 NEAREST NEIGHBOR CLASSIFIER ANALYSIS

Nearest neighbor classifiers are memory-based and require no classifier to be fit. Given a query point x_0 , the k training points $x_{(i)}$, $i = 1, \dots, k$ closest in distance to x_0 are

identified and then the point X_0 is classified using the k nearest neighbors. Ties can be broken at random. In some embodiments, Euclidean distance in feature space is used to determine distance as:

$$d(x_0) = \|x_0 - x_o\|$$

- 5 Typically, when the nearest neighbor algorithm is used, the expression data used to compute the linear discriminant is standardized to have mean zero and variance 1. In the present invention, the members of the training population are randomly divided into a training set and a test set. For example, in one embodiment, two thirds of the members of the training population are placed in the training set and one third of the members of the training population are placed in the test set. A select combination of genes described in the present invention represents the feature space into which members of the test set are plotted. Next, the ability of the training set to correctly characterize the members of the test set is computed. In some embodiments, nearest neighbor computation is performed several times for a given combination of genes of the present invention. In each iteration of the computation, the members of the training population are randomly assigned to the training set and the test set. Then, the quality of the combination of genes is taken as the average of each such iteration of the nearest neighbor computation.

- The nearest neighbor rule can be refined to deal with issues of unequal class priors, differential misclassification costs, and feature selection. Many of these refinements involve some form of weighted voting for the neighbors. For more information on nearest neighbor analysis, see Duda, *Pattern Classification*, Second Edition, 2001, John Wiley & Sons, Inc; and Hastie, 2001, *The Elements of Statistical Learning*, Springer, New York.

5.14.6 LINEAR DISCRIMINANT ANALYSIS

- Linear discriminant analysis (LDA) attempts to classify a subject into one of two categories based on certain object properties. In other words, LDA tests whether object attributes measured in an experiment predict categorization of the objects. LDA typically requires continuous independent variables and a dichotomous categorical dependent variable. In the present invention, the expression values for the select combinations of genes described in the present invention across a subset of the training population serve as the requisite continuous independent variables. The trait subgroup classification of each of the members of the training population serves as the dichotomous categorical dependent variable.

LDA seeks the linear combination of variables that maximizes the ratio of between-group variance and within-group variance by using the grouping information. Implicitly, the linear weights used by LDA depend on how the expression of a molecular marker across the training set separates in the two groups (*e.g.*, a group that has osteoarthritis and a group that does not have osteoarthritis) and how this gene expression correlates with the expression of other genes. In some embodiments, LDA is applied to the data matrix of the N members in the training sample by K genes in a combination of genes described in the present invention. Then, the linear discriminant of each member of the training population is plotted. Ideally, those members of the training population representing a first subgroup (*e.g.* those subjects that do not have osteoarthritis) will cluster into one range of linear discriminant values (*e.g.*, negative) and those member of the training population representing a second subgroup (*e.g.* those subjects that have osteoarthritis) will cluster into a second range of linear discriminant values (*e.g.*, positive). The LDA is considered more successful when the separation between the clusters of discriminant values is larger. For more information on linear discriminant analysis, see Duda, *Pattern Classification*, Second Edition, 2001, John Wiley & Sons, Inc; and Hastie, 2001, *The Elements of Statistical Learning*, Springer, New York; Venables & Ripley, 1997, *Modern Applied Statistics with s-plus*, Springer, New York.

5.14.7 QUADRATIC DISCRIMINANT ANALYSIS

Quadratic discriminant analysis (QDA) takes the same input parameters and returns the same results as LDA. QDA uses quadratic equations, rather than linear equations, to produce results. LDA and QDA are interchangeable, and which to use is a matter of preference and/or availability of software to support the analysis. Logistic regression takes the same input parameters and returns the same results as LDA and QDA.

5.14.8 SUPPORT VECTOR MACHINES

In some embodiments of the present invention, support vector machines (SVMs) are used to classify subjects using geneset genetic information. SVMs are a relatively new type of learning algorithm. See, for example, Cristianini and Shawe-Taylor, 2000, *An Introduction to Support Vector Machines*, Cambridge University Press, Cambridge, Boser *et al*, 1992, "A training algorithm for optimal margin classifiers, in *Proceedings of the 5th Annual ACM Workshop on Computational Learning Theory*, ACM Press, Pittsburgh, PA, pp. 142-152; Vapnik, 1998, *Statistical Learning Theory*, Wiley, New York. When used for classification, SVMs separate a given set of binary labeled training data with a hyper-

plane that is [a maximal distance from each point using a fitting algorithm. For cases in which no linear separation is possible, SVMs can work in combination with the technique of 'kernels', which automatically realizes a non-linear mapping to a feature space. The hyper-plane found by the SVM in feature space corresponds to a non-linear decision
 5 boundary in the input space.

In one approach, when a SVM is used, the gene expression data is standardized to have mean zero and unit variance and the members of a training population are randomly divided into a training set and a test set. For example, in one embodiment, two thirds of the members of the training population are placed in the training set and one third of the
 10 members of the training population are placed in the test set. The expression values for a combination of genes is used to train the SVM. Then the ability for the trained SVM to correctly classify members in the test set is determined. In some embodiments, this computation is performed several times for a given combination of molecular markers. In each iteration of the computation, the members of the training population are randomly
 15 assigned to the training set and the test set. Then, the quality of the combination of molecular markers is taken as the average of each such iteration of the SVM computation. For more information on SVMs, see Duda, *Pattern Classification*, Second Edition, 2001, John Wiley & Sons, Inc.; Hastie, 2001, *The Elements of Statistical Learning*, Springer, New York; and Furey *et al*, 2000, *Bioinformatics* 16, 906-914.

20 5.14.9 DECISION TREES

In some embodiments of the present invention, decision trees are used to classify subjects using expression data for combinations of genes. Decision tree algorithms belong to the class of supervised learning algorithms. The aim of a decision tree is to induce a classifier (a tree) from real-world example data. This tree can be used to classify unseen
 25 examples which have not been used to derive the decision tree.

A decision tree is derived from training data. An example contains values for the different attributes and what class the example belongs. In the present invention, the training data is expression data for a combination of genes across the training population.

The following algorithm describes a decision tree derivation:

30 Tree(Examples, Class, Attributes)

Create a root node

If all Examples have the same Class value, give the root this label

Else if Attributes is empty label the root according to the most common value

Else begin

Calculate the information gain for each attribute

Select the attribute A with highest information gain and make this the root attribute

For each possible value, v, of this attribute

- 5 Add a new branch below the root, corresponding to $A = v$
- Let $\text{Examples}(v)$ be those examples with $A = v$
- If $\text{Examples}(v)$ is empty, make the new branch a leaf node labeled with the most common value among Examples
- Else let the new branch be the tree created by
- 10 $\text{Tree}(\text{Examples}(v), \text{Class}, \text{Attributes} - \{A\})$
- end

A more detailed description of the calculation of information gain is shown in the following. If the possible classes v_i of the examples have probabilities $P(v_i)$ then the information content I of the actual answer is given by:

$$15 \quad I(P(v_1), \dots, P(v_n)) = \sum_{i=1}^n -P(v_i) \log_2 P(v_i)$$

The I- value shows how much information we need in order to be able to describe the outcome of a classification for the specific dataset used. Supposing that the dataset contains p positive (e.g. has osteoarthritis) and n negative (e.g. healthy) examples (e.g. individuals), the information contained in a correct answer is:

$$20 \quad I\left(\frac{p}{p+n}, \frac{n}{p+n}\right) = -\frac{p}{p+n} \log_2 \frac{p}{p+n} - \frac{n}{p+n} \log_2 \frac{n}{p+n}$$

where \log_2 is the logarithm using base two. By testing single attributes the amount of information needed to make a correct classification can be reduced. The remainder for a specific attribute A (e.g. a gene) shows how much the information that is needed can be reduced.

$$25 \quad \text{Remainder}(A) = \sum_{i=1}^v \frac{p_i + n_i}{p+n} I\left(\frac{p_i}{p_i + n_i}, \frac{n_i}{p_i + n_i}\right)$$

"v" is the number of unique attribute values for attribute A in a certain dataset, "i" is a certain attribute value, "pi" is the number of examples for attribute A where the

classification is positive (e.g. cancer), " n_i " is the number of examples for attribute A where the classification is negative (e.g. healthy).

The information gain of a specific attribute A is calculated as the difference between the information content for the classes and the remainder of attribute A:

$$Gain(A) = I\left(\frac{p}{p+n}, \frac{n}{p+n}\right) - \text{Remainder}(A)$$

The information gain is used to evaluate how important the different attributes are for the classification (how well they split up the examples), and the attribute with the highest information.

In general there are a number of different decision tree algorithms, many of which are described in Duda, *Pattern Classification*, Second Edition, 2001, John Wiley & Sons, Inc. Decision tree algorithms often require consideration of feature processing, impurity measure, stopping criterion, and pruning. Specific decision tree algorithms include, but are not limited to classification and regression trees (CART), multivariate decision trees, ID3, and C4.5.

In one approach, when a decision tree is used, the gene expression data for a select combination of genes described in the present invention across a training population is standardized to have mean zero and unit variance. The members of the training population are randomly divided into a training set and a test set. For example, in one embodiment, two thirds of the members of the training population are placed in the training set and one third of the members of the training population are placed in the test set. The expression values for a select combination of genes described in the present invention is used to construct the decision tree. Then, the ability for the decision tree to correctly classify members in the test set is determined. In some embodiments, this computation is performed several times for a given combination of molecular markers. In each iteration of the computation, the members of the training population are randomly assigned to the training set and the test set. Then, the quality of the combination of molecular markers is taken as the average of each such iteration of the decision tree computation.

5.14.10 EVOLUTIONARY METHODS

Inspired by the process of biological evolution, evolutionary methods of classifier design employ a stochastic search for an optimal classifier. In broad overview, such methods create several classifiers - a population - from a combination of genes described

in the present invention. Each classifier varies somewhat from the other. Next, the classifiers are scored on expression data across the training population. In keeping with the analogy with biological evolution, the resulting (scalar) score is sometimes called the fitness. The classifiers are ranked according to their score and the best classifiers are retained (some portion of the total population of classifiers). Again, in keeping with biological terminology, this is called survival of the fittest. The classifiers are stochastically altered in the next generation - the children or offspring. Some offspring classifiers will have higher scores than their parent in the previous generation, some will have lower scores. The overall process is then repeated for the subsequent generation: The classifiers are scored and the best ones are retained, randomly altered to give yet another generation, and so on. In part, because of the ranking, each generation has, on average, a slightly higher score than the previous one. The process is halted when the single best classifier in a generation has a score that exceeds a desired criterion value. More information on evolutionary methods is found in, for example, Duda, Pattern Classification, Second Edition, 2001, John Wiley & Sons, Inc.

5.14.11 BAGGING, BOOSTING AND THE RANDOM SUBSPACE METHOD

Bagging, boosting and the random subspace method are combining techniques that can be used to improve weak classifiers. These techniques are designed for, and usually applied to, decision trees. In addition, Skurichina and Duin provide evidence to suggest that such techniques can also be useful in linear discriminant analysis.

In bagging, one samples the training set, generating random independent bootstrap replicates, constructs the classifier on each of these, and aggregates them by a simple majority vote in the final decision rule. See, for example, Breiman, 1996, Machine Learning 24, 123-140; and Efron & Tibshirani, An Introduction to Bootstrap, Chapman & Hall, New York, 1993.

In boosting, classifiers are constructed on weighted versions of the training set, which are dependent on previous classification results. Initially, all objects have equal weights, and the first classifier is constructed on this data set. Then, weights are changed according to the performance of the classifier. Erroneously classified objects (molecular markers in the data set) get larger weights, and the next classifier is boosted on the reweighted training set. In this way, a sequence of training sets and classifiers is obtained, which is then combined by simple majority voting or by weighted majority voting in the

final decision. See, for example, Freund & Schapire, "Experiments with a new boosting algorithm," Proceedings 13th International Conference on Machine Learning, 1996, 148-156.

To illustrate boosting, consider the case where there are two trait extremes exhibited by the population under study, extreme phenotype 1 (e.g., severe osteoarthritis), and extreme phenotype 2 (e.g., no osteoarthritis). Given a vector of predictor molecular marker X selected in step 214, a classifier G(X) produces a prediction taking one of the type values in the two value set: {extreme phenotype 1, extreme phenotype 2}. The error rate on the training sample is

$$\overline{\text{err}} = \frac{1}{N} \sum_{i=1}^N I(y_i \neq G(x_i))$$

where N is the number of subjects in the training set (the sum total of the subjects that have either extreme phenotype 1 or extreme phenotype 2). For example, if there are 49 organisms that have severe osteoarthritis and 72 organisms that have no osteoarthritis under study, N is 121.

A weak classifier is one whose error rate is only slightly better than random guessing. In the boosting algorithm, the weak classification algorithm is repeatedly applied to modified versions of the data, thereby producing a sequence of weak classifiers $G_m(x)$, $m = 1, 2, \dots, M$. The predictions from all of the classifiers in this sequence are then combined through a weighted majority vote to produce the final prediction:

$$G(x) = \text{sign} \left(\sum_{m=1}^M \alpha_m G_m(x) \right)$$

Here $\alpha_1, \alpha_2, \dots, \alpha_M$ are computed by the boosting algorithm and their purpose is to weigh the contribution of each respective $G_m(x)$. Their effect is to give higher influence to the more accurate classifiers in the sequence.

The data modifications at each boosting step consist of applying weights W_1, W_2, \dots, w_n to each of the training observations (x_i, y_i) , $i = 1, 2, \dots, N$. Initially all the weights are set to $w_i = 1/N$, so that the first step simply trains the classifier on the data in the usual manner. For each successive iteration $m = 2, 3, \dots, M$ the observation weights are individually modified and the classification algorithm is reapplied to the weighted observations. At step m , those observations that were misclassified by the classifier G_m .

$i(x)$ induced at the previous step have their weights increased, whereas the weights are decreased for those that were classified correctly. Thus as iterations proceed, observations that are difficult to correctly classify receive ever-increasing influence. Each successive classifier is thereby forced to concentrate on those training observations that are missed by previous ones in the sequence.

The exemplary boosting algorithm is summarized as follows:

1. Initialize the observation weights $w_i = 1/N$, $i = 1, 2, \dots, N$.

2. For $m = 1$ to M :

(a) Fit a classifier $G_m(x)$ to the training set using weights w_i .

10 (b) Compute

$$\text{err}_m = \frac{\sum_{i=1}^N w_i I(y_i \neq G_m(x_i))}{\sum_{i=1}^N w_i}$$

(c) Compute $\alpha_m = \log((1 - \text{err}_m) / \text{err}_m)$.

(d) Set $w_i \leftarrow w_i \cdot \exp(-\alpha_m I(y_i \neq G_m(x_i)))$, $i = 1, 2, \dots, N$.

15 3. Output $G(x) = \text{sign} \left[\sum_{m=1}^M \alpha_m G_m(x) \right]$

In the algorithm, the current classifier $G_m(x)$ is induced on the weighted observations at line 2a. The resulting weighted error rate is computed at line 2b. Line 2c calculates the weight α_m given to $G_m(x)$ in producing the final classifier $G(x)$ (line 3). The individual weights of each of the observations are updated for the next iteration at line 2d. Observations misclassified by $G_m(x)$ have their weights scaled by a factor $\exp(\alpha_m)$, increasing their relative influence for inducing the next classifier $G_{m+1}(x)$ in the sequence. In some embodiments, modifications of the Freund and Schapire, 1997, Journal of Computer and System Sciences 55, pp. 119-139, boosting method are used. See, for example, Hasti et al., The Elements of Statistical Learning, 2001, Springer, New York, Chapter 10. In some embodiments, boosting or adaptive boosting methods are used.

25 In some embodiments, modifications of Freund and Schapire, 1997, Journal of Computer and System Sciences 55, pp. 119-139, are used. For example, in some embodiments, feature preselection is performed using a technique such as the nonparametric scoring methods of Park et al., 2002, Pac. Symp. Biocomput. 6, 52-63. Feature preselection is a form of dimensionality reduction in which the genes that discriminate between classifications the best are selected for use in the classifier. Then, the LogitBoost procedure introduced by Friedman et al., 2000, Ann Stat 28, 337-407 is

used rather than the boosting procedure of Freund and Schapire. In some embodiments, the boosting and other classification methods of Ben-Dor et al., 2000, Journal of Computational Biology 7, 559-583 are used in the present invention. In some
5 embodiments, the boosting and other classification methods of Freund and Schapire, 1997, Journal of Computer and System Sciences 55, 119-139, are used.

 In the random subspace method, classifiers are constructed in random subspaces of the data feature space. These classifiers are usually combined by simple majority voting in the final decision rule. See, for example, Ho, "The Random subspace method for
10 constructing decision forests," IEEE Trans Pattern Analysis and Machine Intelligence, 1998; 20(8): 832-844.

5.14.12 OTHER MATHEMATICAL MODELS

 The pattern classification and statistical techniques described above are merely examples of the types of classifiers that can be used to construct a classifier. Moreover, combinations of the techniques described above can be used. Some combinations, such as
15 the use of the combination of decision trees and boosting, have been described. However, many other combinations are possible. In addition, other techniques in the art such as Projection Pursuit and Weighted Voting can be used to construct classifiers in instances of
step 216.

5.15 IMPLEMENTING THE INVENTION

20 The present invention provides methods and systems for screening molecular markers to identify classifiers and/or for identifying classifiers for a trait and allows for the configuration of classifiers based on combinations of a large number of molecular markers. The invention provides a selection process for reducing the potential large number of candidate molecular markers and/or combinations thereof down to a manageable number
25 which can be evaluated in one or more mathematical models to derive one or more classifiers.

 Some embodiments of the invention are preferably implemented on a computer system having a processor and a memory unit. The embodiments may be implemented as one or more software programs operating on a general purpose computer, such as a
30 personal computer or workstation, or as dedicated special purpose hardware components. The invention allows for an identification of classifiers while reducing the system requirements. It provides techniques for consideration of a large number of potential

molecular markers in the classifier identification process with limited memory and computational requirements.

Some embodiments of the invention provide a data-driven selection of a subset of the candidate molecular markers based on their discrimination ability. Thus, it becomes possible to start out with a large group of potentially interesting molecular markers and to automatically prune the set of candidate molecular markers so that the computer system can handle the classifier identification process more efficiently, i.e. within less processing time and less memory space. This becomes more important as combinations of the molecular markers are generated in the classifier identification process and mathematical models are applied to each combination to derive classifiers, which may be a computationally expensive process, in particular when iterative techniques are applied, such as clustering, decision trees, neural networks, or evolutionary methods. Since the possible number of combinations grows almost exponentially with the number of candidate molecular markers, the processing time for the classifier evaluation become a serious problem. The pruning of candidate molecular markers allows for the consideration of two, three, four and more combinations of candidate molecular markers as basis for the classifiers. It enables the evaluation of a large number of classifiers based on molecular markers showing a promising discrimination ability and supersedes a computer resource consuming evaluation of classifiers based on molecular markers which are likely not contributing to the final trait discrimination. Thus, the invention can be implemented on a computer having less computational power and memory while the quality of the derived classifiers is maintained. On the other hand, the invention allows for the consideration of more molecular markers, which are potentially interesting for a given application (trait), with the same available system resources resulting in a possible higher classification accuracy for the derived classifiers.

The invention further provides a data-driven selection of the derived classifiers to remove classifiers and/or molecular markers which do not significantly contribute to the trait discrimination in the later application phase. This automatic pruning step evaluates the discrimination power of the individual classifiers to reduce the necessary system requirements of a diagnostic system applying the selected classifiers for the wide variety of possible medical applications. Thus, a diagnostic system configured with the identified classifiers needs less computational power and memory and may be implemented on a smaller, less expensive device. This becomes more important when the applied mathematical models are more complex and powerful. Examples of complex classifiers are

described in section 5.14 of this description and include, e.g. neural networks, nearest neighbor classifiers, decision tress, etc. The invention enables the operation of optimized combinations of complex classifiers on diagnostic devises with limited resources.

5.16 EMBODIMENTS OF THE INVENTION

5

6. EXAMPLES

Computer systems, computer program products, methods, and kits for providing health care have been disclosed. What follows are select examples that illustrate the utility and value of the present invention.

10

6.1 EVALUATING OSTEOARTHRITIS CLASSIFIERS USING ROC CURVES

This example demonstrates the use of an embodiment of the invention to identify individuals with mild osteoarthritis. Osteoarthritis is a form of degenerative joint disease that involves the deterioration of and changes to the cartilage and bone. In response to inflammation in and about the joint, the body responds with bony recalcification around the joint structure. This process can be slow and gradual with minimal outward symptoms, or more rapidly progressive with significant pain and discomfort. Arthritic changes can occur in response to infection and injury of the joint as well.

Step 202 - generation of a training population.

Blood samples were taken from 44 test individuals not having any symptoms of osteoarthritis and 50 individuals having mild osteoarthritis using the methods described in Section 5.2. A molecular marker profile resulting in data for molecular marker products of the entire human genome was measured from each of these samples. This gene expression profile data together with knowledge of which subjects have osteoarthritis and which do not constitutes the training population 44. The 44 test individuals that do not have any symptoms of osteoarthritis constitute one trait subgroup within the training population and the 50 individuals having mild osteoarthritis constitute another trait subgroup within the training population.

Steps 204-218.

The training population collected in step 202 was used in order to identify combinations of genes that can serve as a classifier to differentiate mild osteoarthritis from non-osteoarthritis. Thus, the classifiers developed in this example are designed to yield a positive score when they predict that a subject has mild osteoarthritis and a negative score when they predict that the subject is in the control population. Using the approach

described in Section 5.1, two specific classifiers were developed: 100000252 and 100000511. Classifier 100000252 comprises six genes and has the format:

$$\text{SCORE} = -1.839 + 0.8 \cdot \text{HSPCA} - 1.5525 \cdot \text{IKBKAP} + 1.10184 \cdot \text{IL13RA1} + 0.78923 \cdot \text{LAMC1} - 1.3974 \cdot \text{MAFB} + 1.0602 \cdot \text{PF4}.$$

5 Classifier 100000511 comprises nine genes and has the format:

$$\text{SCORE} = -4.3754 + 0.10276 \cdot \text{EGRI} - 1.1697 \cdot \text{G2AN} + 0.88767 \cdot \text{HSPCA} - 0.55785 \cdot \text{IKBKAP} + 0.94015 \cdot \text{IL13RA1} + 0.67515 \cdot \text{LAMC1} - 1.5068 \cdot \text{MAFB} + 1.0798 \cdot \text{PF4} + 0.4007 \cdot \text{TNFAIP6}.$$

Here, EGRI, G2AN, HSPCA, IKBKAP, IL13RA1, LAMC1, MAFB, and
10 TNFAIP6 are genes that were identified in step 204 and validated in step 208 (Section 5.1) for their ability to discriminate between subjects that have mild osteoarthritis and subjects that do not have osteoarthritis.

Step 220.

To judge which classifier is more suitable as a classifier for mild osteoarthritis, a
15 ROC curve was computed for both classifiers using the gene expression data from the 44 test individuals not having any symptoms of osteoarthritis and the 50 individuals having mild osteoarthritis. The results of the ROC computation are illustrated in Fig. 8. The area under each ROC was computed. From this computation, it was determined that the area under the ROC curve corresponding to classifier 100000252 was 0.863 whereas the area
20 under the ROC curve corresponding to classifier 100000511 was 0.8169.

Step 224.

In some embodiments, a classifier can be constructed that includes both classifiers 100000252 and 100000511 using the voting methods described in Section 5.1. In
alternative embodiments, classifier 100000252 is selected to serve as a classifier for mild
25 osteoarthritis because it generated a larger area under the ROC curve corresponding to the classifier when tested against the training population.

6.2 IDENTIFIED MOLECULAR MARKERS AND MOLECULAR MARKER DATA MEASUREMENT TECHNIQUES

Molecular markers useful for input into one or more steps of the invention and
30 techniques for measuring data values of such molecular markers, can be found in United States patent application serial No. 10/601,518, filed June 20, 2003, United States patent application serial No. 10/802,875, filed March 12, 2004, United States patent application serial No. 10/809,675, filed March 25, 2004, United States patent application serial No. 10/268,730, filed October 9, 2002, United States patent application serial No. 09/477,148,

filed January 4, 2000, United States patent application serial No. 60/1 15,125, filed Jan. 6, 1999; and United States patent application serial No. 60/581,977, filed June 21, 2004 each of which is hereby incorporated herein by reference in its entirety.

6.3 CONSTRUCTION OF CLASSIFIERS FOR MANIC DEPRESSION SYNDROME

This example demonstrates the use of the claimed invention to identify biomarkers to differentiate manic depression syndrome from non manic depression syndrome and use of same. As used herein, "manic depression syndrome" (MDS) refers to a mood disorder characterized by alternating mania and depression.

Step 202.

Blood samples were taken from patients who were diagnosed with manic depression as defined herein, in each case, the diagnosis of manic depression was corroborated by a skilled Board certified physician. Molecular marker data was measured for each of the molecular markers of the entire human genome using blood samples from individuals who were identified as having manic depression as described herein and individuals not having manic depression. Molecular marker data for both trait subgroups were compared and gene expression profiles for each trait subpopulation compared using commercially available GeneSpring™ softwares. Hybridizations to create the gene expression profiles were done using Affymetrix® GeneChip® platforms (U133A and U133 Plus 2.0) as described herein (data not shown). Samples from patients were clustered into two trait subgroups. The first trait subgroup included patients who have manic depression and the second trait subgroup included patients who do not have manic depression (i.e., control individuals).

Step 204.

The Wilcoxon Mann Whitney rank sum test was used to identify molecular marker data that could discriminate between the control and diseased trait subgroups with a p value of < 0.05 .

Step 206.

Molecular markers were selected from those identified with p value of < 0.05 and the ability to discriminate between the control and diseased trait subgroups were confirmed using quantitative RT-PCR.

Steps 214-218.

Eight candidate molecular markers were chosen and an exhaustive analysis of all possible combinations of said molecular markers were considered. Molecular marker data

for each of the eight candidate molecular markers was obtained for each member of the training population and logistic regression applied to the molecular marker data so as to develop multiple classifiers. Each classifier was ranked on the basis of area under the curve and those classifiers with an ROC of greater than 0.9 chosen.

5 **6.4 CONSTRUCTION OF CLASSIFIERS FOR PREDICTING
RESPONSE TO TREATMENT**

This example demonstrates the use of an embodiment of the invention to identify a classifier for predicting the response of a subject to treatment.

Step 202.

10 Blood samples are taken from patients (for example patients with a disease) who are going to enter into treatment (for the disease), or who are already undergoing treatment, but at a timepoint before being able to determine how the patients will respond to treatment. In one embodiment, blood samples are taken from patients who are about to enter into a clinical trial for a new treatment, or who are in the early stages of a clinical
15 trial. Preferably blood samples are processed so as to preserve the RNA and/or the protein products of the molecular markers. More preferably the blood samples are processed immediately, within 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 10 hours, 12, hours, 18 hours or 24 hours from having taken the blood samples from the patients.

Subsequent to when the blood samples are taken, patients continue to be monitored
20 for response to treatment using traditional diagnostic methods and grouped into trait subgroups on the basis of the response to treatment. For example, trait subgroups can include patients with a positive response and no negative side effects, patients with a positive response and mild side effects, patients with a negative response, patients with a toxic response, and the like. In some embodiments, the evaluation of response to treatment
25 can take days, weeks, months or years. In some embodiments, data as described in step 202 of Section 5.1 and Section 5.3 is obtained upon processing of the blood sample. In other embodiments, data of step 202 is obtained only after a determination of response to treatment has been made. In all cases, molecular marker data is obtained from a blood sample taken at a timepoint prior to being able to determine response to treatment. Once
30 trait subgroups are identified on the basis of response to treatment, gene expression profiles of blood samples of each trait subgroup are compared using GeneSpring™ software analysis. Hybridizations to create the gene expression profiles are done using Affymetrix® GeneChip® platforms (U133A and U133 Plus 2.0) platforms (U133A and U133 Plus 2.0) as described herein. Samples from patients are clustered into two trait

subgroups on the basis of the response to treatment. For example, one trait subgroup demonstrates a positive response to treatment whereas the second trait subgroup demonstrates a toxic response to treatment.

Step 204.

5 The Wilcoxon Mann Whitney rank sum test is used to identify candidate molecular markers by identifying molecular marker data that discriminates between the response and non-response trait subgroups with a p value of < 0.05 to obtain candidate molecular markers.

Step 206.

10 Additional molecular marker data for the candidate molecular markers are obtained using quantitative RT-PCR. Some candidate molecular markers are removed at this point should the quantitative RT-PCR data not confirm the ability of each candidate molecular marker to discriminate as between the response trait subgroups.

Steps 214 - 218.

15 Candidate molecular marker combinations are chosen and an exhaustive analysis of all possible combinations of molecular markers are tested. To test all possible combinations of molecular markers, logistic regression is applied to the molecular marker data so as to develop multiple classifiers. Each classifier is ranked on the basis of area under the curve using the training population. Those classifiers ranking with an ROC area
20 under curve of greater than 0.9 are further evaluated using a scoring population which is not the training population. Note that the blood samples used for the scoring population are obtained at the same time point as the blood samples used for the training population (e.g., at a time prior to being able to determine response to treatment).

6.5 CONSTRUCTION OF CLASSIFIERS FOR DETERMINING A TRAIT OF INTEREST

6.5.1

This example demonstrates the selection of the composition of the training population and the trait subgroups of the training population so as to result in classifiers which are useful to predict disease.

Step 202.

In order to predict disease, blood samples are taken from patients at a time when said patients are disease free. Preferably blood samples are processed so as to preserve the

RNA and/or the protein products of all molecular markers of the entire genome of said individual. More preferably the blood samples are processed immediately, within 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 10 hours, 12, hours, 18 hours or 24 hours from having taken the blood samples from the patients.

5 Subsequent to when the blood samples are taken, patients continue to be monitored for development of said disease using traditional diagnostic methods. At a given time point, two trait subgroups are identified, namely individuals who develop said disease of interest and individuals who do not develop said disease of interest. In some embodiments, the timepoint at which trait subgroups are identified can take days, weeks, 10 months or years. In some embodiments, data as described in step 202 of Section 5.1 and Section 5.3 is obtained upon processing of the blood sample. In other embodiments, data of step 202 is obtained only after a determination of trait subgroups has been made. In all cases, data is obtained from a blood sample taken at a timepoint prior to being able to determine disease. Once trait subgroups are identified gene expression profiles of the 15 molecular marker data of the molecular marker products from the blood samples of each trait subgroup are compared using GeneSpring™ software analysis. Hybridizations to create the gene expression profiles are done using Affymetrix® GeneChip® platforms (U133A and U133 Plus 2.0) platforms (U133A and U133 Plus 2.0) as described herein and candidate molecular markers are identified where the molecular marker data is able to 20 differentiate as between said two trait subgroups with a p value of <0.05. Said candidate molecular markers are subsequently processed as described in steps 206 to 226 to identify classifiers and molecular markers capable of predicting disease.

6.5.2

25 This example demonstrates the selection of the composition of the training population and the trait subgroups of the training population so as to result in classifiers which are useful to determine treatment compliance.

Step 202.

30 In order to determine treatment compliance, blood samples are taken from patients who are complying with said treatment of interest and patients who are not complying with said treatment. Preferably blood samples are processed so as to preserve the RNA and/or the protein corresponding to molecular markers of the entire genome of said individual. More preferably the blood samples are processed immediately, within 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 10 hours, 12, hours, 18 hours or 24 hours from having taken the blood samples from the patients.

Molecular marker data is obtained as described in step 202 of Section 5.1 and Section 5.3 upon processing of the blood sample. Hybridizations to create the gene expression profiles are done using Affymetrix® GeneChip® platforms (U133A and U133 Plus 2.0) platforms (U133A and U133 Plus 2.0) as described herein and candidate molecular markers are identified which differentiate as between patients who comply with said treatment of interest as compared with patients who do not comply with said treatment of interest with a p value of 0.05. Said candidate molecular markers are subsequently processed as described in steps 206 to 226 to identify classifiers and molecular markers capable of determining treatment compliance.

6.5.3

This example demonstrates the selection of the composition of the training population and the trait subgroups of the training population so as to result in classifiers which are useful to predict reoccurrence of disease.

Step 202.

In order to predict reoccurrence of disease, blood samples are taken from patients, all of whom have had a disease of interest, at a time when all of said patients are disease free. Preferably blood samples are processed so as to preserve the RNA and/or the protein corresponding to molecular markers of the entire genome of said individual. More preferably the blood samples are processed immediately, within 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 10 hours, 12 hours, 18 hours or 24 hours from having taken the blood samples from the patients.

Subsequent to when the blood samples are taken, patients continue to be monitored for reoccurrence of said disease using traditional diagnostic methods. At a given time point, two trait subgroups are identified, namely individuals who develop reoccurrence of said disease of interest and individuals who do not develop said disease of interest. In some embodiments, the timepoint at which trait subgroups are identified can take days, weeks, months or years. In some embodiments, data as described in step 202 of Section 5.1 and Section 5.3 is obtained upon processing of the blood sample. In other embodiments, data of step 202 is obtained only after a determination of trait subgroups has been made. In all cases, data is obtained from a blood sample taken at a timepoint prior to being able to determine reoccurrence of disease. Once trait subgroups are identified molecular marker data of the molecular marker products from the blood samples of each trait subgroup are compared using GeneSpring™ software analysis. Hybridizations to create the gene expression profiles are done using Affymetrix® GeneChip® platforms

(U133A and U133 Plus 2.0) platforms (U133A and U133 Plus 2.0) as described herein and candidate molecular markers are identified whose molecular marker data differentiates as between said two trait subgroups with a p value of <0.05 . Said candidate molecular markers are subsequently processed as described in steps 206 to 226 to identify classifiers and molecular markers capable of predicting reoccurrence of disease.

6.5.4

This example demonstrates the use of classifiers used in series so as to diagnose a patient with a stage of disease, for example a specific stage of osteoarthritis.

We have identified previously four different stages of osteoarthritis; namely mild osteoarthritis, moderate osteoarthritis, marked osteoarthritis and severe osteoarthritis (see for example PCT patent application WO02070737 Entitled "Compositions and Methods relating to osteoarthritis")

In some instances it is useful to determine which stage of osteoarthritis an individual has, and more importantly to confirm that said patient does not have any other stage of osteoarthritis. For example if an individual has changed their lifestyle and lost weight -to determine whether the osteoarthritis has regressed.

Classifiers of the invention are able to differentiate as between two subgroups. As such—multiple classifiers are required to specifically stage an individual.

A first classifier is developed which differentiates as between osteoarthritis and non-osteoarthritis. As described, a training population is selected comprised of two trait subgroups where said first trait subgroup is comprised of individuals having osteoarthritis and the second trait subgroup comprised of individuals not having osteoarthritis. Identification of candidate molecular markers which differentiate as between these two trait subgroups are identified as per step 202 and subsequently processed as described in steps 206 to 226 to identify classifiers and molecular markers capable of differentiating between osteoarthritis and non-osteoarthritis.

Similarly classifiers are identified which are capable of differentiating between (a) mild osteoarthritis and moderate osteoarthritis (b) moderate osteoarthritis and marked osteoarthritis (c) marked osteoarthritis and severe osteoarthritis.

In order to diagnose an individual as having marked osteoarthritis and not having mild osteoarthritis or severe osteoarthritis, a series of tests are applied. First a classifier which determines whether said patient has osteoarthritis or not is applied. Assuming said patient has osteoarthritis, a classifier is applied which determines whether said patient has either mild osteoarthritis or marked osteoarthritis. Assuming said patient has marked

osteoarthritis,"! classiKeFis applied to determine whether said patient has marked osteoarthritis or severe osteoarthritis. The result of these series of classifiers can determine that said patient has marked osteoarthritis and does not have any other stage of osteoarthritis.

5

6.6 CONSTRUCTION OF CLASSIFIERS FOR DETERMINING A TRAIT OF INTEREST (OR DIFFERENTIATING BETWEEN TWO TRAITS OF INTEREST) USING THE MOLECULAR MARKERS IDENTIFIED IN ONE OF THE DISCLOSED TABLES

10 While the examples described below suggest selection of molecular markers prior to generating all combinations of classifiers from the disclosed Tables can be based on a specific measure of statistical significance (p value) as disclosed for each molecular marker (see Table G and Table H) it must be appreciated that the selection of molecular markers may also be based on any other method disclosed in this application, such as

15 differential fold change or even a combination of selection of p value and differential fold change. A skilled person in the art will recognize these other methods can also be used so as to permit the selection of subsets of the molecular markers to derive lists for which a reasonable number of combinations can be tested within the limits of the computer processing capacity. The skilled person will be able to transfer the details given in this

20 section for examples based on a p value evaluation to carry out the selection based on other disclosed selection methods or selection methods known to him.

TABLE F

Selected Table	Trait of Interest	Recommended Training Population	
		Key Trait of Members of Trait Subgroup A	Key Trait of Members of Trait Subgroup B
Table 1A	Osteoarthritis and Hypertension	Members have both Osteoarthritis and Hypertension	Members have neither Osteoarthritis nor Hypertension
Table 1B	Osteoarthritis and Obesity	Members have both Osteoarthritis and Obesity	Members have neither Osteoarthritis nor Obesity
Table 1C	Osteoarthritis and Allergies	Members have both Osteoarthritis and Allergies	Members have neither Osteoarthritis nor Allergies
Table 1D	Osteoarthritis and	Members have both	Members have

	Systemic Steroids	Osteoarthritis and are taking Systemic Steroids	neither Osteoarthritis nor are taking Steroids
Table 1E	Hypertension	Members have Hypertension	Members do not have Hypertension
Table 1F	Obesity	Members are Obese	Members are not Obese
Table 1G	Hypertension	Members have Hypertension.	Members do not have Hypertension
		Members have Hypertension and Osteoarthritis.	Members do not have Osteoarthritis
Table 1H	Hypertension and Osteoarthritis	Members have Hypertension and Osteoarthritis	Members do not have either Hypertension or Osteoarthritis
Table 1I	Obesity	Members are Obese	Members are not Obese
		Members have Obesity and Osteoarthritis	Members do not have Osteoarthritis.
Table 1J	Obesity and Osteoarthritis	Members have Osteoarthritis and are also Obese	Members do not have either Osteoarthritis and are not Obese
Table 1K	Allergies	Members have Allergies	Members do not have Allergies
		Members have Allergies and Osteoarthritis	Members do not have Osteoarthritis
Table 1L	Allergies and Osteoarthritis	Members have Allergies and Osteoarthritis	Members do not have either Allergies or Osteoarthritis
Table 1M	Systemic Steroids	Members have been taking Systemic Steroids	Members have not been taking Systemic Steroids
		Members have been taking Systemic Steroids and have Osteoarthritis	Members do not have Osteoarthritis.
Table 1N	Systemic Steroids and Osteoarthritis	Members have Osteoarthritis and have been taking Systemic Steroids	Members do not have Osteoarthritis and have not been taking systemic Steroids
Table 1O	Taking Birth Control	Members taking Birth Control	Members not taking Birth Control
	Taking Prednisone	Members taking Prednisone	Members not taking Prednisone
	Taking Hormone Replacement	Members taking Hormone	Members not taking Hormone

	Therapy	Replacement Therapy	Replacement Therapy
Table 1P	Type II Diabetes	Members have Type II Diabetes	Members do not have Type II Diabetes
Table 1Q	Hyperlipidemias	Members have Hyperlipidemia	Members do not have Hyperlipidemia
Table 1R	Lung Disease	Members have Lung Disease	Members do not have Lung Disease
Table 1S	Bladder Cancer	Members have Bladder Cancer	Members do not have Bladder Cancer
Table 1T	Early Stage Bladder Cancer	Members have Early Stage Bladder Cancer	Members do not have Bladder Cancer
		Members have Early Stage Bladder Cancer	Members do not have Early Stage Bladder Cancer
	Late Stage Bladder Cancer	Members have Late Stage Bladder Cancer	Members do not have Bladder Cancer
		Members have Late Stage Bladder Cancer	Members do not have Late Stage Bladder Cancer
Table 1U	Coronary Artery Disease (CAD)	Members have CAD	Members do not have CAD
Table 1V	Rheumatoid Arthritis (RA)	Members have RA	Members do not have RA
Table 1W	Rheumatoid Arthritis (RA)	Members have RA	Members do not have RA
Table 1X	Depression	Members have Depression	Members do not have Depression
Table 1Y	Stage of Osteoarthritis - Mild	Members have Mild OA	Members do not have OA
	Stage of Osteoarthritis - Moderate	Members have Moderate OA	Members do not have OA
	Stage of Osteoarthritis - Marked	Members have Marked OA	Members do not have OA
	Stage of Osteoarthritis - Severe	Members have Severe OA	Members do not have OA
Table 1Z	Liver Cancer	Members have Liver Cancer	Members do not have Liver Cancer
Table 1Z(b)	Liver Cancer	Members have Liver Cancer	Members do not have Liver Cancer
Table 1AA	Schizophrenia	Members have Schizophrenia	Members do not have Schizophrenia
Table 1AB	Chagas Disease	Members have Chagas Disease	Members do not have Chagas Disease
Table 1AC	Asthma	Members have	Members have OA

		Asthma and OA	
Table 1AD	Asthma	Members have Asthma	Members do not have Asthma
Table 1AE	Lung Cancer	Members have Lung Cancer	Members do not have Lung Cancer
Table 1AG	Hypertension	Members have Hypertension	Members do not have Hypertension
Table 1AH	Obesity	Members have Obesity	Members do not have Obesity
Table 1AI	Ankylosing Spondylitis	Members have Ankylosing Spondylitis	Members do not have Ankylosing Spondylitis
Table 2	Osteoarthritis	Members have Osteoarthritis	Members do not have Osteoarthritis
Table 3A	Schizophrenia or Manic Depression Syndrome (MDS)	Members have Schizophrenia	Members have MDS
Table 3B	Hepatitis or Liver Cancer	Members have Hepatitis	Members have Liver Cancer
Table 3C	Bladder Cancer or Liver Cancer	Members have Bladder Cancer	Members have Liver Cancer
Table 3D	Bladder Cancer or Testicular Cancer	Members have Bladder Cancer	Members have Testicular Cancer
Table 3E	Testicular Cancer or Kidney Cancer	Members have Testicular Cancer	Members have Kidney Cancer
Table 3F	Liver Cancer or Stomach Cancer	Members have Liver Cancer	Members have Stomach Cancer
Table 3G	Liver Cancer or Colon Cancer	Members have Liver Cancer	Members have Colon Cancer
Table 3H	Stomach Cancer or Colon Cancer	Members have Stomach Cancer	Members have Colon Cancer
Table 3I	Rheumatoid Arthritis or Osteoarthritis	Members have Rheumatoid Arthritis	Members have Osteoarthritis
Table 3K	Chagas Disease or Heart Failure	Members have Chagas Disease	Members have Heart Failure
Table 3L	Chagas Disease or Coronary Artery Disease	Members have Chagas Disease	Members have CAD
Table 3N	Coronary Artery Disease or Heart Failure	Members have CAD	Members have Heart Failure
Table 3P	Asymptomatic Chagas or Symptomatic Chagas	Members have Asymptomatic Chagas	Members have Symptomatic Chagas
Table 3Q	Alzheimer's or Schizophrenia	Members have Alzheimer's	Members have Schizophrenia
Table 3R	Alzheimer's or Manic Depression Syndrome	Members have Alzheimer's	Members have Manic Depression

Table 4A	Osteoarthritis	Members have Osteoarthritis	Members do not have Osteoarthritis
Table 4B	Osteoarthritis	Members have Osteoarthritis	Members do not have Osteoarthritis
Table 4C	Mild Osteoarthritis	Members have Mild Osteoarthritis	Members do not have Osteoarthritis
Table 4D	Mild Osteoarthritis	Members have Mild Osteoarthritis	Members do not have Osteoarthritis
Table 4E	Moderate Osteoarthritis	Members have Moderate Osteoarthritis	Members do not have Osteoarthritis
Table 4F	Moderate Osteoarthritis	Members have Moderate Osteoarthritis	Members do not have Osteoarthritis
Table 4G	Marked Osteoarthritis	Members have Marked Osteoarthritis	Members do not have Osteoarthritis
Table 4H	Marked Osteoarthritis	Members have Marked Osteoarthritis	Members do not have Osteoarthritis
Table 4I	Severe Osteoarthritis	Members have Severe Osteoarthritis	Members do not have Osteoarthritis
Table 4J	Severe Osteoarthritis	Members have Severe Osteoarthritis	Members do not have Osteoarthritis
Table 4K	Mild Osteoarthritis or Moderate Osteoarthritis	Members have Mild Osteoarthritis	Members have Moderate Osteoarthritis
Table 4L	Mild Osteoarthritis or Moderate Osteoarthritis	Members have Mild Osteoarthritis	Members have Moderate Osteoarthritis
Table 4M	Mild Osteoarthritis or Marked Osteoarthritis	Members have Mild Osteoarthritis	Members have Marked Osteoarthritis
Table 4N	Mild Osteoarthritis or Marked Osteoarthritis	Members have Mild Osteoarthritis	Members have Marked Osteoarthritis
Table 4O	Mild Osteoarthritis or Severe Osteoarthritis	Members have Mild Osteoarthritis	Members have Severe Osteoarthritis
Table 4P	Mild Osteoarthritis or Severe Osteoarthritis	Members have Mild Osteoarthritis	Members have Severe Osteoarthritis
Table 4Q	Moderate Osteoarthritis or Marked Osteoarthritis	Members have Moderate Osteoarthritis	Members have Marked Osteoarthritis
Table 4R	Moderate Osteoarthritis or Marked Osteoarthritis	Members have Moderate Osteoarthritis	Members have Marked Osteoarthritis
Table 4S	Moderate	Members have	Members have

	Osteoarthritis or Severe Osteoarthritis	Moderate Osteoarthritis	Severe Osteoarthritis
Table 4T	Moderate Osteoarthritis or Severe Osteoarthritis	Members have Moderate Osteoarthritis	Members have Severe Osteoarthritis
Table 4U	Marked Osteoarthritis or Severe Osteoarthritis	Members have Marked Osteoarthritis	Members have Severe Osteoarthritis
Table 4V	Marked Osteoarthritis or Severe Osteoarthritis	Members have Marked Osteoarthritis	Members have Severe Osteoarthritis
Table 5A	Psoriasis	Members have Psoriasis	Members do not have Psoriasis
Table 5B	Thyroid Disorder	Members have Thyroid Disorder	Members do not have Thyroid Disorder
Table 5C	Irritable Bowel Syndrome	Members have Irritable Bowel Syndrome	Members do not have Irritable Bowel Syndrome
Table 5D	Osteoporosis	Members have Osteoporosis	Members do not have Osteoporosis
Table 5E	Migraine Headaches	Members have Migraine Headaches	Members do not have Migraine Headaches
Table 5F	Eczema	Members have Eczema	Members do not have Eczema
Table 5G	NASH	Members have NASH	Members do not have NASH
Table 5H	Alzheimer's	Members have Alzheimers'	Members do not have Alzheimers'
Table 5I	Manic Depression Syndrome	Members have Manic Depression Syndrome	Members do not have Manic Depression Syndrome
Table 5J	Crohn's Colitis	Members have Crohn's Colitis	Members do not have Crohn's Colitis
Table 5K	Chronic Cholecystitis	Members have Chronic Cholecystitis	Members do not have Chronic Cholecystitis
Table 5L	Heart Failure	Members have Heart Failure	Members do not have Heart Failure
Table 5M	Cervical Cancer	Members have Cervical Cancer	Members do not have Cervical Cancer
Table 5N	Stomach Cancer	Members have Stomach Cancer	Members do not have Stomach Cancer
Table 5O	Kidney Cancer	Members have Kidney Cancer	Members do not have Kidney Cancer
Table 5P	Testicular Cancer	Members have Testicular Cancer	Members do not have Testicular

			Cancer
Table 5Q	Colon Cancer	Members have Colon Cancer	Members do not have Colon Cancer
Table 5R	Hepatitis B	Members have Hepatitis B	Members do not have Hepatitis B
Table 5S	Pancreatic Cancer	Members have Pancreatic Cancer	Members do not have Pancreatic Cancer
Table 5T	Asymptomatic Chagas	Members have Asymptomatic Chagas	Members do not have Asymptomatic Chagas
Table 5U	Symptomatic Chagas	Members have Symptomatic Chagas	Members do not have Symptomatic Chagas
Table 5V	Bladder Cancer	Members have Bladder Cancer	Members do not have Bladder Cancer
Table 6A	Cancer	Members have Cancer	Members do not have Cancer
Table 6B	Cardiovascular Disease	Members have Cardiovascular Disease	Members do not have Cardiovascular Disease
Table 6C	Neurological Disorders	Members have a Neurological Disorder	Members do not have a Neurological Disorder
Table 7A	Celebrex® or Other Cox Inhibitor	Members taking Celebrex®	Members taking non Celebrex® Cox Inhibitor
Table 7B	Celebrex®	Members taking Celebrex®	Members not taking Celebrex®
Table 7C	Vioxx®	Members taking Vioxx®	Members not taking Vioxx®
Table 7D	Vioxx® or Other Cox Inhibitor	Members taking Vioxx®	Members taking non Vioxx® Cox Inhibitor
Table 7E	NSAIDS	Members taking NSAIDS	Members not taking NSAIDS
Table 7F	Cortisone	Members taking Cortisone	Members not taking Cortisone
Table 7G	Visco Supplement	Members taking Visco Supplement	Members not taking Visco Supplement
Table 7H	Lipitor®	Members taking Lipitor®	Members not taking Lipitor®
Table 7I	Smokers	Members are Smokers	Members are not Smokers

CONSTRUCTION OF CLASSIFIERS FOR DETERMINING A TRAIT OF INTEREST Steps 202-204

- In order to identify useful classifiers for a trait of interest, for example mild
- 5 osteoarthritis, one or more of the tables listed in Table F above which have the same

recommended training population can be used. Thus for example, for mild osteoarthritis, one can select one or more of Tables IY; 4C; 4D. These molecular markers listed resulted from application of Steps 202-204 as outlined in Figure 2A as more fully described for each Table herein. Once one or more Tables have been selected, it is helpful to select a subset of the molecular markers in the Table or Tables before proceeding to step 206. For example, combining Tables IY, 4C and 4D and selecting molecular markers where the molecular marker data demonstrates an ability to differentiate as between the two trait subgroups with a p value of less than 0.0001 results in 212 molecular markers. Note that p values resulting from the molecular marker data for each molecular marker identified in any of Tables 1A to 7I can be found in Tables 8A or Tables 8B below.

Table 8A identifies molecular markers via the Clone ID of the probe used to hybridize to the molecular marker products. The Clone ID corresponds to the Clone ID found in tables 1A; 1AC; 1B; 1C; 1D; 1E; 1F; 1G; 1H; 1I; U; 1K; 1L; 1M; 1N; 10; 1P; 1Q; 1R; 1V; 1X; 1Y; 1Z; 2; 4A; 4C; 4E; 4G; 4I; 4K; 4M; 4O; 4Q; 4S; 4V; 7A; 7B; 7C; 7D; 7E; 7H; and 7I (ie those Tables generated using the ChondroChip™ as outlined herein). Table 8A then identifies the corresponding Table in which the molecular marker is identified via said Clone ID. Finally the p value of the molecular marker data obtained using the ChondroChip™ is listed. Note that Table 8A is sorted first by Table number and then by p value.

Table 8B identifies molecular markers via the Affymetrix® Spot ID of the probe pair used to hybridize to the molecular marker products. The Affy Spot ID corresponds to the Affy Spot ID found in tables Tables 1AA; 1AB; 1AD; 1AE; 1AG; 1AH; 1AT; 1S; 1T; 1U; 1W; 1Z(b); 3A; 3B; 3C; 3D; 3E; 3F; 3G; 3H; 3I; 3K; 3L; 3P; 3Q; 3R; 4B; 4D; 4F; 4H; 4J; 4L; 4N; 4P; 4R; 4T; 4V; 5A; 5B; 5C; 5D; 5EE; 5F; 5G; 5H; 5I; 5J; 5K; 5L; 5M; 5N; 5O; 5P; 5Q; 5R; 5S; 5T; 5U; 5V; 6A; 6B; 6C; 7F; and 7G (ie those Tables generated using the Affymetrix™ Gene Chip as outlined herein). Table 8B then identifies the corresponding Table in which the molecular marker is identified via said Affymetrix® Spot ID. Finally the p value of the molecular marker data obtained using the ChondroChip™ is listed. Note that Table 8B is sorted first by Table number and then by p value.

Step 206

For the 212 selected molecular markers - a training population is chosen having two trait subgroups where the two trait subgroups are outlined in Table F above as corresponding to the Tables used to select the molecular markers, thus in this example, the

first trait subgroup is members having mild osteoarthritis and the second trait subgroup is members not having osteoarthritis. A blood sample from each member of the training population is obtained and processed using techniques as described herein and mRNA isolated. The resulting mRNA is reverse transcribed using ABFs High Capacity cDNA Archive Kit and the cDNA is then used for quantitative RT-PCR so as to collect molecular marker data for application to a logistic regression model. Amplification primers are designed for each of the 212 molecular markers. Preferably primers are chosen which amplify across an intron junction. Quantitative Real Time PCR is performed using Qiagen's QuantiTect™ Sybr Green RT-PCR kit and data corresponding to the level of RNA for each of the molecular markers

Steps 214 - 218.

From the 212 candidate molecular markers selected, an exhaustive analysis of all possible combinations of molecular markers using the molecular marker data obtained using quantitative RT-PCR data is tested using logistic regression so as to develop multiple classifiers. Each classifier is ranked on the basis of area under the curve using the training population. Those classifiers ranking with an ROC area under curve of greater than 0.8 are further evaluated using a scoring population which is not the training population. Those classifiers resulting in an ROC area under the curve of greater than 0.7 as determined using the scoring population are selected. Each of the selected classifiers is comprised of a combination of molecular markers from one of Tables IY, 4C and 4D.

USE OF THE SELECTED CLASSIFIER TO DIAGNOSE AN INDIVIDUAL AS HAVING MILD OSTEOARTHRITIS.

Any of the selected classifiers can be used to diagnose an individual as having mild osteoarthritis. A blood sample from a test individual is processed using techniques as described herein to isolate mRNA. mRNA is reverse transcribed using ABFs High Capacity cDNA Archive Kit and the cDNA is then used for quantitative RT-PCR. Amplification primers are designed for each of the molecular markers in the classifier selected which amplify across an intron junction. Quantitative Real Time PCR is performed using Qiagen's QuantiTect™ Sybr Green RT-PCR kit and data corresponding to the level of RNA for each of the molecular markers of the selected classifier obtained. The data is then used in conjunction with the logistic regression classifier so as to convert the data resulting from the Quantitative RT-PCR into a single number. If the number is greater than 0 - the test individual is diagnosed as having mild osteoarthritis is the number is less than 0 the test individual is diagnosed as not having osteoarthritis.

7. REFERENCES CITED

All references cited herein are incorporated herein by reference in their entirety and for all purposes to the same extent as if each individual publication or patent or patent application was specifically and individually indicated to be incorporated by reference in
5 its entirety for all purposes.

The present invention can be implemented as a computer program product that comprises a computer program mechanism embedded in a computer readable storage medium. For instance, the computer program product could contain the program modules shown in Fig. 1. These program modules may be stored on a CD-ROM, DVD, magnetic
10 disk storage product, or any other computer readable data or program storage product. The software modules in the computer program product can also be distributed electronically, via the Internet or otherwise, by transmission of a computer data signal (in which the software modules are embedded) on a carrier wave.

Many modifications and variations of this invention can be made without departing
15 from its spirit and scope, as will be apparent to those skilled in the art. The specific embodiments described herein are offered by way of example only, and the invention is to be limited only by the terms of the appended claims, along with the full scope of equivalents to which such claims are entitled.

Table 8A

Clone ID	TableNo.	p-value	Clone ID	TableNo.	P Value
miob8143	IA	2.14e-05	fcrb4231	IA	3.784194e-03
ncrc5844	IA	1.135 β e-04	seob0755	IA	3.784194e-03
hfcr3149	IA	1.30609e-04	seoc1023	IA	3.784194e-03
fcrb3330	IA	1.71826e-04	fcrb4 995	IA	4.164516e-03
ncrc9772	IA	1.71826e-04	fcrb6031	IA	4.164516e-03
fcrb2162	IA	2.24449e-04	fcrb6896	IA	4.164516e-03
ncrb8343	IA	2.55864e-04	fcrcl689	IA	4.164516e-03
fcrb4226	IA	2.91185e-04	fcrc6228	IA	4.164516e-03
seoc6182	IA	2.91185e-04	ncr7672	IA	4.164516e-03
seoa3408	IA	3.75265e-04	ncrc1885	IA	4.164516e-03
fcrb6191	IA	4.80528e-04	ncrc3544	IA	4.164516e-03
mioc1028	IA	4.80528e-04	seoa2381	IA	4.164516e-03
seob2966	IA	5.4249e-04	seob9872	IA	4.164516e-03
seoc0775	IA	5.4249e-04	fcrb2090	IA	4.577725e-03
fcrcl834	IA	7.73507e-04	hfcr0489	IA	4.577725e-03
ncrc3257	IA	7.88975e-04	miocl4 48	IA	4.577725e-03
ncrc2472	IA	8.68041e-04	ncr7813	IA	4.577725e-03
mioc3930	IA	1.216349e-03	ncrc9712	IA	4.577725e-03
ncr0847	IA	1.216349e-03	ncrc9855	IA	4.577725e-03
miob8947	IA	1.253946e-03	seoa0256	IA	4.577725e-03
fcrb5214	IA	1.357347e-03	seoa3555	IA	4.577725e-03
miob4037	IA	1.512638e-03	mioa0601	IA	4.9929e-03
miob8932	IA	1.512638e-03	fcrb2713	IA	5.026153e-03
fcrc0166	IA	1.683445e-03	mioa1704	IA	5.026153e-03
miob8249	IA	1.683445e-03	miob6419	IA	5.026153e-03
seob3485	IA	1.683445e-03	raioc2997	IA	5.026153e-03
seob7929	IA	1.683445e-03	mioc3127	IA	5.026153e-03
seob0154	IA	1.871074e-03	ncr7904	IA	5.026153e-03
fcrc6345	IA	2.076917e-03	ncrc5088	IA	5.026153e-03
mioa5059	IA	2.076917e-03	seoa2641	IA	5.026153e-03
fcrb4378	IA	2.302455e-03	seoa3359	IA	5.026153e-03
mioc4066	IA	2.302455e-03	seob5069	IA	5.026153e-03
ncrc5091	IA	2.302455e-03	seob5213	IA	5.026153e-03
mioc0162	IA	2.452971e-03	ncr1948	IA	5.341175e-03
fcrc5516	IA	2.549264e-03	fcrb9324	IA	5.512248e-03
miob4308	IA	2.549264e-03	fcrc6990	IA	5.512248e-03
seob1319	IA	2.549264e-03	mioc1598	IA	5.512248e-03
fcr1997	IA	2.819017e-03	seob4752	IA	5.512248e-03
fcr4471	IA	2.819017e-03	mioa0528	IA	6.038584e-03
ncr0615	IA	2.819017e-03	mioc2074	IA	6.038584e-03
ncr9549	IA	2.819017e-03	mioc5703	IA	6.038584e-03
ncrc0729	IA	2.819017e-03	ncrc3391	IA	6.038584e-03
fcrb4572	IA	3.113486e-03	seob0168	IA	6.038584e-03
fcrb9655	IA	3.113486e-03	fcrb4345	IA	6.607863e-03
ncrb6320	IA	3.113486e-03	mioa2377	IA	6.607863e-03
seoa9709	IA	3.113486e-03	mioc1122	IA	6.607863e-03
seoa9870	IA	3.113486e-03	ncrc9637	IA	6.607863e-03
seob2959	IA	3.113486e-03	seob9152	IA	6.607863e-03
seoc1025	IA	3.113486e-03	fcrbl691	IA	7.222914e-03
fcr4214	IA	3.43455e-03	fcrb5928	IA	7.222914e-03
fcrb3897	IA	3.43455e-03	fcrc β 010	IA	7.222914e-03
fcrb6508	IA	3.43455e-03	fcrc6566	IA	7.222914e-03
fcrb7944	IA	3.43455e-03	hfcr4477	IA	7.222914e-03
fcrc2807	IA	3.43455e-03	miob8691	IA	7.222914e-03
ncrc5780	IA	3.43455e-03	mioc2330	IA	7.222914e-03
seoa0429	IA	3.43455e-03	ncr3037	IA	7.222914e-03
seoa1977	IA	3.43455e-03	ncrc9700	IA	7.222914e-03
fcr3559	IA	3.784194e-03	seoa9889	IA	7.222914e-03

se6b8311	IA	7.222914e-03
fcrb7584	IA	7.288639e-03
fcrb5204	IA	7.886697e-03
fcrc5614	IA	7.886697e-03
mioc7331	IA	7.886697e-03
mioc8682	IA	7.886697e-03
ncr0212	IA	7.886697e-03
ncr7292	IA	7.886697e-03
ncrb4331	IA	7.886697e-03
ncrb5595	IA	7.886697e-03
seoa6654	IA	7.886697e-03
seob4545	IA	7.886697e-03
seob8301	IA	7.886697e-03
seoc1664	IA	7.886697e-03
fcr1312	IA	8.602308e-03
fcrb2041	IA	8.602308e-03
mioa5085	IA	8.602308e-03
ncrc8892	IA	8.602308e-03
seoa1584	IA	8.602308e-03
seoa5577	IA	8.602308e-03
seob5319	IA	8.602308e-03
seob6386	IA	8.602308e-03
seob8741	IA	8.602308e-03
fcr1879	IA	9.372976e-03
fcrbl457	IA	9.372976e-03
fcrb3644	IA	9.372976e-03
fcrb4388	IA	9.372976e-03
mioa8851	IA	9.372976e-03
mioa8852	IA	9.372976e-03
mioc1060	IA	9.372976e-03
mioc1910	IA	9.372976e-03
miod6437	IA	9.372976e-03
ncr6072	IA	9.372976e-03
seoa8399	IA	9.372976e-03
seob4197	IA	9.372976e-03
seob9092	IA	9.372976e-03
fcrbl689	IA	0.01
fcrbl990	IA	0.01
fcrb8542	IA	0.01
seoa9705	IA	0.01
seob0688	IA	0.01
seob6279	IA	0.01
seoc3965	IA	0.01
fcr3664	IA	0.01
fcr3717	IA	0.01
fcrb3686	IA	0.01
fcrb5813	IA	0.01
fcrb6012	IA	0.01
fcrb7803	IA	0.01
fcrb8989	IA	0.01
mioc6937	IA	0.01
ncrb8437	IA	0.01
ncrc5631	IA	0.01
seoa2448	IA	0.01
seoa8543	IA	0.01
seob6131	IA	0.01
seob6272	IA	0.01
seob9406	IA	0.01
seob9851	IA	0.01
fcr7042	IA	0.01
fcrb4799	IA	0.01
fcrc6877	IA	0.01
mioa9649	IA	0.01
mioc1125	IA	0.01

miod7421	IA	0.01
ncr001β	IA	0.01
ncrb8385	IA	0.01
ncrc5949	IA	0.01
seoa2805	IA	0.01
seoa6620	IA	0.01
seob3141	IA	0.01
fcr4639	IA	0.01
fcrb3237	IA	0.01
fcrb3244	IA	0.01
fcrb4981	IA	0.01
fcrb5850	IA	0.01
fcrb9686	IA	0.01
fcrb9959	IA	0.01
hfcr1811	IA	0.01
miob3953	IA	0.01
mioc0669	IA	0.01
ncrc2119	IA	0.01
ncrc4575	IA	0.01
ncrc6127	IA	0.01
ncrc6382	IA	0.01
seoc0778	IA	0.01
seoc0924	IA	0.01
ncrc1892	IA	0.01
fcr0593	IA	0.01
fcrb3715	IA	0.01
mioc4420	IA	0.01
miod3920	IA	0.01
ncr6343	IA	0.01
seob5209	IA	0.01
ncrl876	IA	0.01
miod3417	IA	0.01
fcr4212	IA	0.01
fcrb4413	IA	0.01
fcrb5305	IA	0.01
fcrb9253	IA	0.01
hfcr1189	IA	0.01
miod4464	IA	0.01
ncr3614	IA	0.01
ncrc3536	IA	0.01
ncrc9562	IA	0.01
seoa3105	IA	0.01
seob0085	IA	0.01
seob3513	IA	0.01
seob5044	IA	0.01
seob5336	IA	0.01
fcrb4985	IA	0.01
hfcr6634	IA	0.01
mioa5586	IA	0.01
mioa9831	IA	0.01
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ncr4140	IE	8.29e-06	fcrc6976	IE	6.54736e-04
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seoc2447	IE	8.722468e-03	fcrb2639	IE	0.01
miod6364	IE	9.069698e-03	fcrb3896	IE	0.01
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fcrb3702	IE	9.349172e-03	fcrc2082	IE	0.01
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miob 597	IE	9.349172e-03	mioa6428	IE	0.01

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fcrb2424	IE	0.01
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fcrb5892	IE	0.01
fcrb6202	IE	0.01
fcrb6643	IE	0.01
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miob4333	IE	0.01
mioc7986	IE	0.02
fcr3282	IE	0.02
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seoa2775	IE	0.02
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fcrb2166	IE	0.02
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ncrc7049	IE	0.02

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ncrc0849	IE	0.03	miob2656	IE	0.04
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miob5810	IE	0.04	hfcrl5865	IE	0.04
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ncrc6416	IE	0.04	mioa2290	IF	1.00455e-04
ncrc8903	IE	0.04	mioa3987	IF	1.01e-04
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seoa3555	IE	0.04	ncr2363	IF	1.17e-04
seoa5447	IE	0.04	mioc5633	IF	1.43481e-04
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seocl230	IE	0.04	mioa2204	IF	1.61204e-04
mioa1077	IE	0.04	seoa3516	IF	1.61204e-04
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mioc1002	IE	0.04	ncr0132	IF	1.89e-04
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seoc3554	IE	0.04	ncr3598	IF	2.41e-04
fcrc0725	IE	0.04	fcrl3936	IF	2.51e-04
seob9734	IE	0.04	mioa8864	IF	2.53933e-04
ncr2583	IE	0.04	fcrlb1296	IF	2.69e-04
fcrlb177 5	IE	0.04	hfcrl6033	IF	2.73e-04
fcrlb1916	IE	0.04	ncrc3257	IF	2.77e-04
fcrlb8872	IE	0.04	fcrl4214	IF	2.91e-04
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miob0487	IE	0.04	fcrl6748	IF	3.0e-04
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seoc0513	IE	0.04	hfcrl5991	IF	3.11e-04
fcrc2376	IE	0.04	seoa5554	IF	3.36e-04
fcrc2737	IE	0.04	mioa3392	IF	3.68e-04
fcrc4948	IE	0.04	mioa2213	IF	3.85e-04
ncr0451	IE	0.04	fcrlb2784	IF	3.9297e-04
ncr3177	IE	0.04	fcrlb8196	IF	3.9297e-04
ncrc4882	IE	0.04	ncrb8530	IF	4.1e-04
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seob5762	IE	0.04	ncr4975	IF	4.61e-04
fcrl5425	IE	0.04	seob4075	IF	4.85728e-04
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fcrlb7885	IE	0.04	ncr3815	IF	5.17e-04
fcrc4848	IE	0.04	hfcrl5942	IF	5.23e-04
fcrc5007	IE	0.04	ncr8843	IF	5.4e-04
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mioa3987	IE	0.04	fcrlb2334	IF	5.71e-04
miob2067	IE	0.04	hfcrl2275	IF	5.9789e-04
miob2756	IE	0.04	mioa7465	IF	6.04e-04
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mioc7542	IE	0.04	fcrc6542	IF	6.5e-04
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ncrc1247	IE	0.04	seob4105	IF	6.64e-04
ncrc3856	IE	0.04	miobl545	IF	7.28e-04
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miod3132	IG	0.03	seoa1480	IG	0.04
ncr8538	IG	0.03	seoa2899	IG	0.04
ncr8594	IG	0.03	seoa6118	IG	0.04
ncrc0185	IG	0.03	seoa6144	IG	0.04
ncrc1531	IG	0.03	miob8143	IH	9.6e-05
ncrc3324	IG	0.03	fcrb6191	IH	2.18748e-04
seoa4012	IG	0.03	fcrb4995	IH	5.08096e-04
fcrb2462	IG	0.03	hfcrl4477	IH	5.46853e-04
fcrb4360	IG	0.03	miob7209	IH	7.83893e-04
fcrb5948	IG	0.03	fcrb9202	IH	9.02252e-04
miob4760	IG	0.03	ncr2472	IH	9.02252e-04
miob7794	IG	0.03	miob7554	IH	9.67277e-04
mioc4351	IG	0.03	fcrb3330	IH	1.188468e-03
ncr2926	IG	0.03	seoc2191	IH	1.188468e-03
ncrb1365	IG	0.03	miob4308	IH	1.659667e-03
seoa5392	IG	0.03	fcrb2162	IH	2.15038e-03
seob1889	IG	0.03	mioc2451	IH	2.15038e-03
seob4446	IG	0.03	miob8572	IH	2.291704e-03
seoc1278	IG	0.03	fcrc6345	IH	2.441249e-03
fcrb061	IG	0.04	miod1203	IH	2.441249e-03
hfcrl259	IG	0.04	seob3112	IH	2.441249e-03
hfcrl4497	IG	0.04	seoc3965	IH	2.599424e-03
miod7246	IG	0.04	fcrb7944	IH	3.13006e-03
ncr3233	IG	0.04	fcrc2807	IH	3.13006e-03
ncrc5359	IG	0.04	hfcrl811	IH	3.32717e-03

mxod5703	IH	~y.32717e-03
ncr8628	IH	3.986113e-03
ncrb3957	IH	3.986113e-03
seob8741	IH	3.986113e-03
fcr7042	IH	4.487107e-03
mxod7421	IH	5.04282e-03
fcr1984	IH	5.342732e-03
mioc3930	IH	5.342732e-03
mxoc0728	IH	5.989924e-03
seob5478	IH	7.089543e-03
fcrc5614	IH	7.917127e-03
ncrb8343	IH	7.917127e-03
fcr3323	IH	8.361625e-03
fcrbl428	IH	8.827692e-03
mioc0669	IH	8.827692e-03
miod5122	IH	8.827692e-03
ncrc0729	IH	9.316185e-03
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seob2966	IH	0.01
fcrb6031	IH	0.01
fcrc0166	IH	0.01
fcrb2041	IH	0.01
fcrb9680	IH	0.01
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seoc3965	II	5.85651e-04
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fcrc3907	II	1.200832e-03
ncr2484	II	1.200832e-03
seob6395	II	1.200832e-03
fcrb8994	II	1.361048e-03
seoa1747	II	1.44814e-03
mxoa4014	II	1.540198e-03
seoa5986	II	1.848665e-03

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fcrb2759	II	1.963134e-03	miod0126	II	0.01
mioc5113	II	1.963134e-03	hfcr5691	II	0.01
fcrb6005	II	2.083894e-03	mioa0247	II	0.01
miob0178	II	2.083894e-03	mioa8818	II	0.01
seoa3322	II	2.083894e-03	mioa8899	II	0.01
ncrc2831	II	2.486944e-03	ncrc3072	II	0.01
ncr5713	II	2.63595e-03	ncrc4033	II	0.01
ncrb8538	II	2.792845e-03	fcrb7183	II	0.01
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mioa3913	II	5.433839e-03	seoa2801	II	0.01
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fcrb8208	II	5.730596e-03	seob4333	II	0.01
miob0795	II	5.730596e-03	seob5021	II	0.01
fcrb6829	II	6.041484e-03	fcrb0131	II	0.01
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fcrb6728	II	6.367062e-03	fcrb2318	II	0.01
fcrc2314	II	6.367062e-03	fcrb4918	II	0.01
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miob1789	II	7.437766e-03	ncr8112	II	0.01
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seob1052	II	8.236011e-03	fcrb2113	II	0.01
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miod0978	II	8.662397e-03	mioa2478	II	0.01
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fcrbl441	II	9.107857e-03	miod5894	II	0.01
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mioc3306	IJ	4.377496e-03
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ncrc3624	IJ	5.150669e-03
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hfcrc0734	IJ	5.730596e-03
seoa4436	IJ	5.730596e-03
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mioc2074	IJ	7.064602e-03
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mioc0978	IR 6.234951e-03	seoa9373	IR	0.01
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mioc4348	IR 6.431377e-03	fcrb5840	IR	0.01
ncr9123	IR 6.431377e-03	miobl059	IR	0.01
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fcrc7180	IR 6.981505e-03	miod2834	IR	0.01
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seoc0438	IR 7.267233e-03	fcrb2800	IR	0.01
seoc2131	IR 7.267233e-03	fcr5470	IR	0.01
mioc2887	IR 7.625229e-03	mioc1978	IR	0.01
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fcrb3550	IR 8.107057e-03	fcrb7430	IR	0.01
hfcr5383	IR 8.196224e-03	fcrb8196	IR	0.01
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miob9495	IR 8.352358e-03	ncr5890	IR	0.01
mioa3428	IR 8.522496e-03	ncr7097	IR	0.01
fcr3033	IR 8.949232e-03	ncrb7635	IR	0.01
miod4083	IR 8.954907e-03	ncrc0090	IR	0.01
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seoa5396	IR 9.201027e-03	seoc5627	IR	0.01
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ncrb8751	IR 9.226752e-03	fcr5092	IR	0.01
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fcr3269	IR 9.538296e-03	fcrbl977	IR	0.01
fcr2986	IR 9.702695e-03	mioc0371	IR	0.01
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ncrc4076	IR 9.933523e-03	ncrc1003	IR	0.01
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seoa0238	IR	0.04	seob2775	IV	1.28e-05
fcrc0559	IR	0.04	fcrb5214	IV	2.5e-05
ncrc0803	IR	0.04	fcrb8489	IV	2.68e-05
mioc6925	IR	0.04	mioc6211	IV	3.64e-05
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ncrc0863	IR	0.04	hfcr3180	IV	4.19e-05
miob47 60	IR	0.04	fcrb2256	IV	4.65e-05
seob5954	IR	0.04	ncrc9436	IV	5.3e-05
seoa5302	IR	0.04	fcrb4981	IV	5.58e-05
fcrb6301	IR	0.04	seoa9997	IV	6.35e-05
ncrc0922	IR	0.04	seoc0009	IV	6.35e-05
fcrc5007	IR	0.04	miob9805	IV	7.39e-05
fcrc1313	IR	0.04	seoa0099	IV	8.58e-05
mioc0240	IR	0.04	seob4197	IV	8.72e-05
miod7099	IR	0.04	fcr5836	lv	9.95e-05
ncrc0341	IR	0.04	fcrc5604	IV	1.06e-04
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fcr2182	IR	0.04	fcrb4781	IV	1.15081e-04
fcrbl604	IR	0.04	fcrb2137	IV	1.21e-04
fcrbl854	IR	0.04	seob2937	IV	1.23e-04
fcrc4005	IR	0.04	fcrb3966	IV	1.27e-04
hfcr3067	IR	0.04	seob2938	IV	1.52e-04
hfcr3070	IR	0.04	hfcr2287	IV	1.53205e-04
hfcr4170	IR	0.04	miod.4564	IV	1.53205e-04
hfcr4275	IR	0.04	seoal460	IV	1.53205e-04
mioa2165	IR	0.04	ncrc5019	IV	1.72e-04
mioa5902	IR	0.04	hfcr5969	IV	1.76303e-04
mioa8452	IR	0.04	mioa2993	IV	2.02535e-04
mioa8594	IR	0.04	miod5505	IV	2.02535e-04
miob5119	IR	0.04	fcrb5123	IV	2.14e-04
miod4407	IR	0.04	hfcr2844	IV	2.22e-04
miod7367	IR	0.04	miod7099	IV	2.26e-04
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ncrb0090	IR	0.04	seoa5652	IV	2.32274e-04
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ncrc6129	IR	0.04	seob7500	IV	2.45e-04
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seoa0064	IR	0.04	seob4306	IV	2.49e-04
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seob8854	IR	0.04	ncrb3980	IV	2.65933e-04
seocl539	IR	0.04	seoa6178	IV	2.65933e-04
seocl764	IR	0.04	seoc3519	lv	2.65933e-04
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seob3485	IR	0.04	mioa3940	IV	3.03966e-04
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ncrb8539	IR	0.04	miod5030	IV	3.46873e-04
ncrc5016	IR	0.04	fcrb3544	IV	3.47e-04
ncr2288	IR	0.04	seocl235	IV	3.49e-04
fcrbl920	IR	0.04	seoa5156	IV	3.5e-04
fcrb8020	IR	0.04	ncrb0164	IV	3.54e-04
seoa4132	IR	0.04	ncrc5025	IV	3.55e-04
seoa9870	IR	0.04	seoa6661	IV	3.66e-04
seob0876	IR	0.04	ncrc4985	IV	3.68e-04
seob2990	IR	0.04	seob7622	IV	3.68e-04

fcr2860	IV	3.7e-04	fcrc7219	IV	5.78978e-04
fcr4128	IV	3.7e-04	mioc2872	IV	5.78978e-04
fcrbl539	IV	3.7e-04	ncrc2857	IV	5.78978e-04
fcrbl990	IV	3.7e-04	seob9851	IV	5.78978e-04
fcrb2330	IV	3.7e-04	mioa8380	IV	5.8e-04
fcrb3119	IV	3.7e-04	hfcr0229	IV	6.04e-04
fcrb5269	IV	3.7e-04	fcrb5351	IV	6.14e-04
fcrb5536	IV	3.7e-04	seob9614	IV	6.2227e-04
fcrb5564	IV	3.7e-04	fcrb5896	IV	6.33e-04
fcrb6251	IV	3.7e-04	fcrc2724	IV	6.33e-04
fcrb6596	IV	3.7e-04	mioc7370	IV	6.33e-04
fcrb6639	IV	3.7e-04	fcr2299	IV	6.35519e-04
fcrb7084	IV	3.7e-04	fcr3053	IV	6.55553e-04
fcrb7693	IV	3.7e-04	fcrc5137	IV	6.55553e-04
fcrc2745	IV	3.7e-04	fcrc5614	IV	6.55553e-04
fcrc2849	IV	3.7e-04	mioa3668	IV	6.55553e-04
fcrc5480	IV	3.7e-04	miod44 93	IV	6.55553e-04
miobl561	IV	3.7e-04	seoa5898	IV	6.55553e-04
miob3456	IV	3.7e-04	seoa8993	IV	6.55553e-04
miob8816	IV	3.7e-04	seoa9160	IV	6.55553e-04
miob9248	IV	3.7e-04	seob2987	IV	6.55553e-04
miob9393	IV	3.7e-04	ncrc3460	IV	7.23e-04
mioc4022	IV	3.7e-04	seob3088	IV	7.31e-04
miod5 622	IV	3.7e-04	fcrb9611	IV	7.41141e-04
ncrl545	IV	3.7e-04	fcrc5107	IV	7.41141e-04
ncrb2288	IV	3.7e-04	mioc3682	IV	7.41141e-04
ncrb7482	IV	3.7e-04	ncrb0749	IV	7.41141e-04
ncrc24 43	IV	3.7e-04	seoc3690	IV	7.41141e-04
ncrc5363	IV	3.7e-04	seocl642	IV	7.46e-04
seoa2209	IV	3.7e-04	fcrb8340	IV	7.85e-04
seoa3230	IV	3.7e-04	miob8825	IV	7.85e-04
seoa7340	IV	3.7e-04	seob9869	IV	8.13e-04
seoa8997	IV	3.7e-04	mioc4161	IV	8.18e-04
seob0303	IV	3.7e-04	fcrb8668	IV	8.3666e-04
seob0763	IV	3.7e-04	fcrc5041	IV	8.3666e-04
seob0872	IV	3.7e-04	fcrc5086	IV	8.3666e-04
seobl426	IV	3.7e-04	fcrc6932	IV	8.3666e-04
seocl6β1	IV	3.7e-04	hfcr3058	IV	8.3666e-04
seoc2221	IV	3.7e-04	seob6437	IV	8.3666e-04
mioa4753	IV	3.89e-04	seob9772	IV	8.3666e-04
fcrb8114	IV	3.91e-04	seoa1318	IV	8.39e-04
seoa1749	IV	3.95201e-04	fcrb9420	IV	8.44e-04
miob9124	IV	4.2e-04	fcrb6715	IV	8.66e-04
fcrc0430	IV	4.28e-04	ncr2575	IV	9.04e-04
seoa5977	IV	4.43e-04	ncrc4875	IV	9.26e-04
fcr1772	IV	4.49e-04	miob9285	IV	9.36e-04
fcrc5577	IV	4.49548e-04	fcrbl684	IV	9.43108e-04
miob7794	IV	4.49548e-04	fcrb2113	IV	9.43108e-04
ncr4030	IV	4.49548e-04	fcrb3686	IV	9.43108e-04
ncrc0576	IV	4.49548e-04	mioa8864	IV	9.43108e-04
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fcrb3680	IV	4.71e-04	miob4574	IV	9.43108e-04
ncr0133	IV	4.83e-04	miob8609	IV	9.43108e-04
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seobl362	IV	5.10569e-04	seob2994	IV	9.76e-04
seoc0149	IV	5.10569e-04	ncrc5039	IV	9.97e-04
fcrc4180	IV	5.27e-04	miocl354	IV	1.061561e-03
fcrb37 63	IV	5.67e-04	ncrb1167	IV	1.073748e-03
ncrc3283	IV	5.67e-04	seoa3895	IV	1.119756e-03
ncrc3358	IV	5.67e-04	seocl307	IV	1.151704e-03
fcr4328	IV	5.78978e-04	seoa1737	IV	1.155164e-03

ncrc9899	IV	1.170347e-03	ncrc3598	IV	1.476515e-03
fcrb3734	IV	1.170828e-03	ncrc4076	IV	1.476515e-03
seob0133	IV	1.181737e-03	ncrc5688	IV	1.476515e-03
miob3618	IV	1.193188e-03	ncrc8988	IV	1.476515e-03
miob7106	IV	1.193188e-03	seoa1856	IV	1.476515e-03
miob7638	IV	1.193188e-03	seoa2768	IV	1.476515e-03
ncrb0328	IV	1.193188e-03	seoa3891	IV	1.476515e-03
ncrc0461	IV	1.193188e-03	seoa4681	IV	1.476515e-03
seob4555	IV	1.193188e-03	seoa5235	IV	1.476515e-03
seoc0034	IV	1.193188e-03	seoa6557	IV	1.476515e-03
mioa0192	IV	1.218739e-03	seoa7517	IV	1.476515e-03
miod4735	IV	1.252641e-03	seoa7530	IV	1.476515e-03
mioa1906	IV	1.255827e-03	seoa8424	IV	1.476515e-03
mioa8973	IV	1.256375e-03	seoa9627	IV	1.476515e-03
seoa9724	IV	1.27253e-03	seoa9792	IV	1.476515e-03
miod.1316	IV	1.29623e-03	seob0370	IV	1.476515e-03
ncrc9159	IV	1.326356e-03	seob1947	IV	1.476515e-03
fcr3001	IV	1.339245e-03	seob5556	IV	1.476515e-03
fcrb8908	IV	1.339245e-03	seoc2722	IV	1.476515e-03
fcrc0591	IV	1.339245e-03	mioa8987	IV	1.493237e-03
fcrc2775	IV	1.339245e-03	miob7319	IV	1.494352e-03
fcrc7056	IV	1.339245e-03	mioa0311	IV	1.501088e-03
hfcr2629	IV	1.339245e-03	mioa2173	lv	1.501088e-03
miob2836	IV	1.339245e-03	ncr4135	IV	1.501088e-03
mioc0669	IV	1.339245e-03	ncrb2544	IV	1.501088e-03
miod3946	IV	1.339245e-03	ncrb4166	IV	1.501088e-03
ncrc0964	IV	1.339245e-03	seoa0536	IV	1.501088e-03
ncrc9052	IV	1.339245e-03	seob8562	IV	1.501088e-03
seoa6172	IV	1.339245e-03	hfcr2670	IV	1.548822e-03
seob4734	IV	1.339245e-03	seoa5554	lv	1.560016e-03
seob7424	IV	1.339245e-03	miob9748	IV	1.637446e-03
fcr4380	IV	1.411242e-03	hfcr0263	lv	1.680177e-03
miod3914	IV	1.413142e-03	hfcr5991	IV	1.680177e-03
miod7081	IV	1.426601e-03	hfcr6613	IV	1.680177e-03
miod1323	IV	1.440791e-03	mioa3913	IV	1.680177e-03
fcrbl922	IV	1.458522e-03	ncrb3768	IV	1.680177e-03
ncr4189	IV	1.458522e-03	fcr0748	IV	1.690349e-03
fcr1756	IV	1.476515e-03	fcrb2292	IV	1.706646e-03
fcr4494	IV	1.476515e-03	fcrc6990	IV	1.722062e-03
fcr4902	IV	1.476515e-03	seob3226	IV	1.79599e-03
fcrbl575	IV	1.476515e-03	seob2728	IV	1.80089e-03
fcrb2483	IV	1.476515e-03	hfcr3444	IV	1.835381e-03
fcrb4470	IV	1.476515e-03	fcr2102	IV	1.844189e-03
fcrb4717	IV	1.476515e-03	fcr385β	IV	1.878078e-03
fcrb7036	IV	1.476515e-03	mioa8952	IV	1.878078e-03
fcrb9454	IV	1.476515e-03	miob8487	IV	1.878078e-03
fcrc0529	IV	1.476515e-03	miod5060	IV	1.878078e-03
fcrc5471	IV	1.476515e-03	miod5123	IV	1.878078e-03
fcrc7222	IV	1.476515e-03	ncr6344	IV	1.878078e-03
hfcr3647	IV	1.476515e-03	seoa0501	IV	1.878078e-03
mioa1626	IV	1.476515e-03	seoa8239	IV	1.878078e-03
miob3307	IV	1.476515e-03	seoc5228	IV	1.878078e-03
miob9734	IV	1.476515e-03	fcrb2800	IV	1.898609e-03
miob9788	IV	1.476515e-03	ncr0634	IV	1.92683e-03
mioc0911	IV	1.476515e-03	seoa4795	IV	1.961962e-03
mioc7364	IV	1.476515e-03	seob7906	IV	1.97218e-03
miod1448	IV	1.476515e-03	fcrc5482	IV	2.019211e-03
miod5092	IV	1.476515e-03	ncrc5230	IV	2.026769e-03
miod7212	IV	1.476515e-03	seoa2805	IV	2.092754e-03
ncr0025	IV	1.476515e-03	fcr2276	IV	2.096472e-03
ncr2967	IV	1.476515e-03	fcrb4345	IV	2.096472e-03
ncr8538	IV	1.476515e-03	fcrc1563	IV	2.096472e-03
ncrb3638	IV	1.476515e-03	fcrc5290	IV	2.096472e-03
ncrc1780	IV	1.476515e-03	fcrc6002	lv	2.096472e-03

mioa 0597	W	2.096472e-03	ncrl712	IV	2.893252e-03
miob7 662	IV	2.096472e-03	ncrc2161	IV	2.893252e-03
miob8320	IV	2.096472e-03	seoa2391	IV	2.893252e-03
miod5010	IV	2.096472e-03	seob4145	IV	2.893252e-03
raiod5256	IV	2.096472e-03	seob9543	IV	2.893252e-03
ncr3825	IV	2.096472e-03	ncr0491	IV	2.89732e-03
ncrb6109	IV	2.096472e-03	seoc1628	IV	3.049387e-03
seoa0207	IV	2.096472e-03	seob6156	IV	3.053926e-03
ncrb2092	IV	2.134438e-03	ncrc4033	IV	3.104345e-03
fcrbl992	IV	2.209983e-03	seob8287	IV	3.182459e-03
seoa3761	IV	2.225021e-03	mioc0302	IV	3.183214e-03
miod4407	IV	2.228163e-03	mioc0621	IV	3.183214e-03
seoa5433	lv	2.23127e-03	ncr2930	IV	3.183214e-03
seoa6620	IV	2.299089e-03	mioc3369	IV	3.203555e-03
fcrb3615	IV	2.337161e-03	ncrc9877	IV	3.209695e-03
fcrb3870	IV	2.337161e-03	fcr1555	IV	3.212904e-03
fcrb5296	IV	2.337161e-03	fcr4129	IV	3.212904e-03
fcrb6508	IV	2.337161e-03	fcrb5467	IV	3.212904e-03
fcrb8119	IV	2.337161e-03	fcrb8236	IV	3.212904e-03
fcrb8215	IV	2.337161e-03	mioa7617	IV	3.212904e-03
fcrc5139	IV	2.337161e-03	miod4269	iv	3.212904e-03
miod5198	IV	2.337161e-03	ncr4656	iv	3.212904e-03
miod6560	IV	2.337161e-03	ncr7923	IV	3.212904e-03
ncr3782	IV	2.337161e-03	ncr8153	IV	3.212904e-03
ncrb0027	IV	2.337161e-03	seoa1599	IV	3.212904e-03
ncrb4477	IV	2.337161e-03	seoa8912	IV	3.212904e-03
ncrb7166	IV	2.337161e-03	mioc1060	IV	3.280556e-03
seoa1173	IV	2.337161e-03	ncrc267 5	iv	3.327646e-03
seobl808	IV	2.337161e-03	irrcr8725	IV	3.386385e-03
seob6206	IV	2.342651e-03	fcrb2713	iv	3.387122e-03
miob9087	IV	2.3779e-03	fcrb6031	IV	3.409264e-03
seob0321	iv	2.379735e-03	hfcr2536	IV	3.539273e-03
miob3594	iv	2.406345e-03	mioa9491	IV	3.555572e-03
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seob0937	IV	2.540573e-03	fcr5536	IV	3.563359e-03
mioc3220	IV	2.541667e-03	fcrbl731	IV	3.563359e-03
fcrb9684	IV	2.564635e-03	mioc2166	IV	3.563359e-03
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fcr3282	IV	2.602068e-03	ncrb2517	IV	3.563359e-03
fcrc0379	IV	2.602068e-03	ncrc6846	IV	3.563359e-03
fcrc4390	IV	2.602068e-03	seoa1992	IV	3.563359e-03
miob5940	IV	2.602068e-03	seoa6152	IV	3.563359e-03
mioc3603	IV	2.602068e-03	seoa8177	IV	3.563359e-03
miod1377	IV	2.602068e-03	seob0058	IV	3.563359e-03
miod5771	IV	2.602068e-03	seob6133	IV	3.563359e-03
miod6324	IV	2.602068e-03	seoc0951	IV	3.563359e-03
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seoa1598	IV	2.602068e-03	seoc4928	IV	3.563359e-03
seob4303	IV	2.602068e-03	ncr4454	IV	3.575847e-03
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ncrc2959	IV	2.604001e-03	seob0085	IV	3.701885e-03
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seoa6131	IV	2.721704e-03	seoa8399	IV	3.841578e-03
fcr4503	IV	2.721914e-03	fcrb5375	IV	3.881761e-03
miod4857	IV	2.870616e-03	miob9533	IV	3.895277e-03
fcr1328	IV	2.893252e-03	ncrb6742	IV	3.895528e-03
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miod00 80	IV	2.893252e-03	mioa8970	IV	3.9471e-03

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mioc7201	IV	3.9471e-03	ncrc4907	IV	4.204851e-03
miod5310	IV	3.9471e-03	ncrc5312	IV	4.204851e-03
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ncrb8239	IV	3.9471e-03	ncrc9328	IV	4.204851e-03
ncrc3855	IV	3.9471e-03	ncrc9910	IV	4.204851e-03
ncrc5376	IV	3.9471e-03	seoa0388	IV	4.204851e-03
ncrc6587	IV	3.9471e-03	seoa3147	IV	4.204851e-03
seoa0256	IV	3.9471e-03	seoa6137	IV	4.204851e-03
seob7278	IV	3.9471e-03	seoa6393	IV	4.204851e-03
miob8754	IV	3.989036e-03	seoa6497	IV	4.204851e-03
seob7584	IV	4.012618e-03	seoa6598	IV	4.204851e-03
ncrb8171	IV	4.135195e-03	seoa6718	IV	4.204851e-03
miocl416	IV	4.135238e-03	seoa7157	IV	4.204851e-03
seob0046	IV	4.135238e-03	seoa8543	IV	4.204851e-03
miob9209	IV	4.157852e-03	seoa9302	IV	4.204851e-03
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fcrb3181	IV	4.204851e-03	seob0154	IV	4.204851e-03
fcrb3201	IV	4.204851e-03	seob0426	IV	4.204851e-03
fcrb4241	IV	4.204851e-03	seobl081	IV	4.204851e-03
fcrb4270	IV	4.204851e-03	seob4645	IV	4.204851e-03
fcrb4294	IV	4.204851e-03	seob5319	IV	4.204851e-03
fcrb4360	IV	4.204851e-03	seob5743	IV	4.204851e-03
fcrb4417	IV	4.204851e-03	seob5748	IV	4.204851e-03
fcrb4963	IV	4.204851e-03	seob5899	IV	4.204851e-03
fcrb5688	IV	4.204851e-03	seob7474	IV	4.204851e-03
fcrb8449	IV	4.204851e-03	seob8483	IV	4.204851e-03
fcrb9371	IV	4.204851e-03	seoc0843	IV	4.204851e-03
fcrc1758	IV	4.204851e-03	fcr0903	IV	4.316218e-03
fcrc2306	IV	4.204851e-03	fcrl499	IV	4.339479e-03
fcrc5583	IV	4.204851e-03	hfcr3143	IV	4.349057e-03
hfcr3022	IV	4.204851e-03	mioc3139	IV	4.358139e-03
mioa1520	IV	4.204851e-03	fcrb3627	IV	4.366761e-03
mioa3321	IV	4.204851e-03	fcrb5503	IV	4.366761e-03
mioa3673	IV	4.204851e-03	fcrc1402	IV	4.366761e-03
mioa4076	IV	4.204851e-03	fcrc5771	IV	4.366761e-03
mioa4564	IV	4.204851e-03	hfcr2895	IV	4.366761e-03
mioa5085	IV	4.204851e-03	miob2668	IV	4.366761e-03
mioa5692	IV	4.204851e-03	miod5672	IV	4.366761e-03
mioa5955	IV	4.204851e-03	ncr4416	IV	4.366761e-03
miob3953	IV	4.204851e-03	ncrb0696	IV	4.366761e-03
miocl229	IV	4.204851e-03	ncrb8585	IV	4.366761e-03
mioc2209	IV	4.204851e-03	seoa1316	IV	4.366761e-03
mioc2348	IV	4.204851e-03	seob4293	IV	4.366761e-03
mioc2451	IV	4.204851e-03	seoc0284	IV	4.366761e-03
mioc2577	IV	4.204851e-03	seoc0742	IV	4.366761e-03
mioc4112	IV	4.204851e-03	seoc4381	IV	4.366761e-03
mioc6374	IV	4.204851e-03	fcrc5007	IV	4.388153e-03
miod2128	IV	4.204851e-03	seob0185	IV	4.388153e-03
miod3160	IV	4.204851e-03	miod7414	IV	4.474241e-03
miod6048	IV	4.204851e-03	seoc2549	IV	4.489551e-03
miod6521	IV	4.204851e-03	mioc4351	IV	4.538604e-03
raiod7324	IV	4.204851e-03	mioc8481	IV	4.678627e-03
ncr0638	IV	4.204851e-03	miod4686	IV	4.740394e-03
ncrl305	IV	4.204851e-03	fcr2972	IV	4.825136e-03
ncrl550	IV	4.204851e-03	fcrc4551	IV	4.825136e-03
ncrb0207	IV	4.204851e-03	fcrc6174	IV	4.825136e-03
ncrb3348	IV	4.204851e-03	hfcr4497	IV	4.825136e-03
ncrb8538	IV	4.204851e-03	miob2285	IV	4.825136e-03
ncrc0539	IV	4.204851e-03	miocl300	IV	4.825136e-03
ncrc1247	IV	4.204851e-03	miod4932	IV	4.825136e-03
ncrc1595	IV	4.204851e-03	ncr8096	IV	4.825136e-03

ncrc9784	IV	4.825136e-03	fcr2935	IV	5.990508e-03
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seob4105	IV	4.825136e-03	seob7309	IV	6.107204e-03
seob7866	IV	4.825136e-03	fcrb7760	IV	6.130086e-03
seoa2899	IV	4.826733e-03	ncrc5088	IV	6.197231e-03
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fcrb5527	IV	4.888032e-03	mioc2021	IV	6.236422e-03
seob5551	IV	4.908034e-03	seoc1804	IV	6.261128e-03
mioa4077	IV	4.909178e-03	miob8463	IV	6.391219e-03
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fcrb3120	IV	5.081016e-03	ncr2484	IV	6.42957e-03
ncrc4448	IV	5.161235e-03	fcrb6301	IV	6.441057e-03
hfcr5865	IV	5.255535e-03	fcr1347	IV	6.462965e-03
fcrb3715	IV	5.312325e-03	fcrb5241	IV	6.462965e-03
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ncrb7027	IV	5.324125e-03	mioa0497	IV	6.462965e-03
fcr1312	IV	5.325183e-03	miob3531	IV	6.462965e-03
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fcr07046	IV	5.325183e-03	ncr2869	IV	6.462965e-03
mioa1473	IV	5.325183e-03	ncr7668	IV	6.462965e-03
mioa9891	IV	5.325183e-03	ncrc0249	iv	6.462965e-03
miob0167	IV	5.325183e-03	ncrc3596	IV	6.462965e-03
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seoa4647	IV	5.325183e-03	seoa6754	IV	6.462965e-03
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seob6096	IV	5.325183e-03	seob0201	IV	6.462965e-03
seoc0369	IV	5.325183e-03	seob3170	IV	6.462965e-03
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seob2685	IV	5.358138e-03	ncrb7350	IV	6.507615e-03
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fcrb6107	IV	5.429727e-03	seoa8300	IV	6.520751e-03
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hfcr2686	IV	5.502936e-03	hfcr3404	IV	6.5438e-03
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mioc2403	IV	5.546828e-03	seob5889	IV	6.584665e-03
mioc2592	IV	5.591403e-03	fcrb1767	IV	6.5959e-03
seoa4066	IV	5.634661e-03	raiob7109	IV	6.695564e-03
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fcr0180	IV	5.870026e-03	fcr5257	IV	7.107476e-03
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miob9678	IV	5.870026e-03	miob1814	IV	7.107476e-03
mioc5182	IV	5.870026e-03	miob4803	IV	7.107476e-03
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ncrb6327	IV	7.17138e-03	fcrb6916	IV	8.677358e-03
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fcr5728	IV	7.78102e-03	fcrb2818	IV	9.351412e-03
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mioc1125	IV	0.01	raioab9128	IV	0.01
mioa3987	IV	0.01	miod1157	IV	0.01
ncr2421	IV	0.01	miod6488	IV	0.01
mioc3605	IV	0.01	ncr0547	IV	0.01
fcrb9017	IV	0.01	ncrl355	IV	0.01
miod5793	IV	0.01	ncr3163	IV	0.01
miob4760	IV	0.01	ncr6415	IV	0.01
ncrc2928	IV	0.01	ncr8866	IV	0.01
fcrb6255	IV	0.01	ncrb0364	IV	0.01
ncrc4440	IV	0.01	ncrc3593	IV	0.01
mioa6543	IV	0.01	seoa4829	IV	0.01
miob3358	IV	0.01	seoa5986	IV	0.01
fcrbl580	IV	0.01	seoa7373	IV	0.01
fcrb3288	IV	0.01	seobl197	IV	0.01
fcrc2705	IV	0.01	seob4075	IV	0.01
hfcr0130	IV	0.01	seob4117	IV	0.01
mioa9062	IV	0.01	seob4270	IV	0.01
mioa9719	IV	0.01	seob9282	IV	0.01
mioa9935	IV	0.01	seocl856	IV	0.01
miobl 811	IV	0.01	seoc4038	IV	0.01
miob3257	IV	0.01	fcrb4271	IV	0.01
miod1056	IV	0.01	seoa9729	IV	0.01
miod4066	IV	0.01	seoa5552	IV	0.01
ncr2176	IV	0.01	fcrc6833	IV	0.01

ncr5651	IV	0.01	seoa0823	IV	0.01
fcrc2429	IV	0.01	seob9435	IV	0.01
ncrc7085	IV	0.01	mioc4280	IV	0.01
mioa3963	IV	0.01	seoc4436	IV	0.01
seoc1906	IV	0.01	mioa5059	IV	0.01
m mioa7 976	IV	0.01	ncrc1606	IV	0.01
seoa9656	IV	0.01	mioc1379	IV	0.01
fcrb3476	IV	0.01	miob0939	IV	0.01
ncrc5091	IV	0.01	fcr2582	IV	0.01
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ncr5709	IV	0.01	fcrb1689	IV	0.01
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seob8194	IV	0.01	fcrb3725	lv	0.01
fcr2908	IV	0.01	fcrb37 60	lv	0.01
fcrb6181	IV	0.01	fcrb4266	lv	0.01
seoa2141	IV	0.01	fcrb4543	IV	0.01
seoa5578	IV	0.01	fcrb5448	IV	0.01
seoa4158	IV	0.01	fcrb5687	IV	0.01
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ncrc6871	IV	0.01	fcrb5774	IV	0.01
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mioa0482	IV	0.01	fcrb8802	IV	0.01
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fcr4639	IV	0.01	fcrc3488	IV	0.01
fcrb2421	IV	0.01	fcrc4054	IV	0.01
fcrb2704	IV	0.01	fcrc5040	IV	0.01
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fcrb3644	IV	0.01	hfcrc3043	IV	0.01
fcrb4504	IV	0.01	hfcrc3441	IV	0.01
fcrb6780	IV	0.01	hfcrc3592	IV	0.01
fcrb8732	IV	0.01	hfcrc5706	IV	0.01
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mioa9630	IV	0.01	mioa4552	IV	0.01
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ncr9933	IV	0.01	miobl506	IV	0.01
ncrc0729	IV	0.01	miob2093	IV	0.01
ncrc0852	IV	0.01	miob4401	IV	0.01
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seob3751	IV	0.01	mioc2806	IV	0.01
seob5658	IV	0.01	mioc4526	IV	0.01
seob9820	IV	0.01	mioc4839	IV	0.01
seoc0416	IV	0.01	mioc7381	IV	0.01
seoc1311	IV	0.01	mioc7863	IV	0.01
seoc4132	IV	0.01	raiocL5884	IV	0.01
fcrb5000	IV	0.01	miod6162	IV	0.01

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ncr3219	IV	0.01	ncrb8220	IV	0.02
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seoa2244	IV	0.01	mioa8096	IV	0.02
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seoa5691	IV	0.01	seoal263	IV	0.02
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seobl007	IV	0.01	ncrc4302	IV	0.02
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seob4079	IV	0.01	fcrc6989	IV	0.02
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seoc1305	IV	0.01	mioa9792	IV	0.02
seoc4609	IV	0.01	miob3690	IV	0.02
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fcrb2133	IV	0.01	mioc7774	IV	0.02
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fcr0529	IV	0.02	seob5395	IV	0.02
fcrbl898	IV	0.02	seoc1996	IV	0.02
fcrb2719	IV	0.02	seoc2487	IV	0.02
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seoc2172	IV	0.03	miob7274	IV	0.03
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seoa3359	IX	5.205548e-03	seoc2191	IX	8.433017e-03
miod4522	IX	5.216795e-03	seoa7295	IX	8.440913e-03
fcrb8187	IX	5.219732e-03	fcrb7118	IX	8.491482e-03
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seocl236	IY	2.97843e-04	fcrc3416	IY	9.27715e-04

mioa2173	IY	9.48317e-04	seob5379	IZ	0.01
ncrc3554	IY	9.48767e-04	seobl513	IZ	0.01
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fcrb6478	IY	9.59421e-04	fcrc6563	IZ	0.01
ncrb6903	IY	1.001243e-03	miob4055	IZ	0.02
fcrb7495	IY	1.020987e-03	seoa7509	IZ	0.02
fcrb8422	IY	1.020987e-03	seoa6573	IZ	0.02
fcrc7219	IY	1.020987e-03	fcrb4696	IZ	0.02
seoa2949	IY	1.020987e-03	seob4805	IZ	0.02
seob4589	IY	1.071134e-03	seocl425	IZ	0.02
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mioc9896	IY	1.350122e-03	mioa4014	IZ	0.02
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miob3560	IY	1.393429e-03	fcrb8504	IZ	0.02
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seob2085	IY	1.615964e-03	mioal062	IZ	0.03
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mioa9179	4A	9.26e-06	mioa2072	4A	4.19e-05
ncr7668	4A	9.93e-06	ncr8588	4A	4.45e-05
seoa1427	4A	1.02e-05	seoa2585	4A	4.45e-05
miob6430	4A	1.1e-05	miod5123	4A	4.5e-05
fcr5075	4A	1.24e-05	seob0248	4A	4.5e-05
fcrbl892	4A	1.24e-05	seob6446	4A	4.81e-05
seoa8916	4A	1.24e-05	seoa4518	4A	5.34e-05
mioc7170	4A	1.26e-05	fcr6235	4A	5.39e-05
seob5240	4A	1.35e-05	hfcrl863	4A	5.39e-05
miob6419	4A	1.37e-05	hfcrl3011	4A	5.39e-05
fcrc0487	4A	1.38e-05	mioa3939	4A	5.39e-05
seoa6133	4A	1.45e-05	mioa6130	4A	5.39e-05
mioc4022	4A	1.52e-05	ncr0258	4A	5.39e-05
mioa1585	4A	1.82e-05	ncr3197	4A	5.39e-05
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seoa0231	4A	1.83e-05	mioc7764	4A	5.6e-05
seoa8754	4A	1.83e-05	ncr0212	4A	5.86e-05
seob4427	4A	1.83e-05	seoc1023	4A	6.08e-05
seob2994	4A	1.84e-05	seob9282	4A	6.18e-05
mioa2319	4A	1.93e-05	fcrl463	4A	6.32e-05
seob6004	4A	2.07e-05	miod1236	4A	6.32e-05
fcr2103	4A	2.09e-05	seob0065	4A	6.32e-05
seoa4461	4A	2.14e-05	seob4029	4A	6.32e-05
mioa0595	4A	2.21e-05	fcrb8504	4A	6.63e-05
seob3518	4A	2.35e-05	seoc2029	4A	6.66e-05
fcrb6939	4A	2.39e-05	seoa3516	4A	7.05e-05
mioa3923	4A	2.39e-05	fcr4831	4A	7.47e-05
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seoa1940	4A	2.66e-05	fcr5559	4A	7.65e-05
seob3189	4A	2.66e-05	fcrb6012	4A	7.67e-05
seob4303	4A	2.66e-05	seob8368	4A	7.81e-05
seobl231	4A	2.9e-05	seoa8566	4A	8.04e-05
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fcrc3750	4A	8.79e-05
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seoa2949	4A	9.47e-05
fcrb3461	4A	9.48e-05
mioa9510	4A	9.71e-05
ncrc5569	4A	9.91e-05
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seob7432	4A	1.03e-04
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mioa5695	4A	1.04e-04
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seob4669	4A	1.04e-04
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seob6368	4A	1.12e-04
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mioa6064	4A	1.17e-04
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mioa0252	4A	1.24e-04
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miob6881	4A	1.37e-04
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seoa9537	4A	1.41e-04
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seob3182	4A	1.41e-04
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seob6835	4A	1.41e-04
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seoc0999	4A	1.46e-04
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seob6131	4A	1.5e-04
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seoa1598	4A	2.2e-04
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seob3119	4A	2.29e-04
miob3348	4A	2.34e-04
mioc0950	4A	2.34e-04
seoa0032	4A	2.34e-04
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hfcrl5604	4A	2.54e-04
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miob4867	4A	2.54e-04
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ncrc6005	4A	2.54e-04
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seoa7443	4A	2.54e-04
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seoa3105	4A	2.79e-04
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ncrc5631	4A	0.02
seob8639	4A	0.02
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seo40429	4A	0.02
fcrb5503	4A	0.02
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fcrbl550	4A	0.02
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fcrb4784	4A	0.02
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seob0918	4A	0.02
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seob0846	4A	0.02
ncrb8747	4A	0.02
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fcrbl691	4A	0.02
miob2743	4A	0.02
mioc3341	4A	0.02
mio46545	4A	0.02
seob0303	4A	0.02
seo45731	4A	0.02
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mioc4839	4A	0.02
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seoa1552	4A	0.02
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seob3918	4A	0.02
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mioc4504	4A	0.02
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seob5144	4A	0.02
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seob7575	4A	0.03
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fcrc0637	4A	0.03
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seob4537	4A	0.03
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mioc3574	4A	0.03
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seoa2402	4A	0.03
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mioc8640	4A	0.03
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seobl191	4A	0.03
seoc2549	4A	0.03
seob0949	4A	0.03
mioc2343	4A	0.03
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seoc4381	4A	0.03
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seob0975	4A	0.04
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seoa8754	4C	3.62e-05
mioa2788	4C	4.4e-05
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seoa8916	4C	5.83e-05
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fcrl1892	4C	7.58e-05
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mioa2319	4C	8.18e-05
seob5240	4C	8.29e-05
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seob0154	4C	1.23e-04
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seoa2585	4C	1.47e-04
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seob6851	4C	1.55e-04
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mioa4009	4C	1.63e-04
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seoc1023	4C	1.66e-04
seoa0959	4C	1.68e-04
seob4029	4C	1.69e-04
mioa4014	4C	1.73e-04
fcrl2704	4C	1.74e-04
seob3189	4C	1.76e-04
miob3953	4C	1.77e-04
miod5651	4C	1.77e-04
seoa2970	4C	1.77e-04
seob5748	4C	1.79e-04
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seob1231	4C	1.85e-04
miod5505	4C	1.89e-04
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fcr2607	4C	2.23e-04
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seoc4960	4C	2.65e-04
miob6223	4C	2.67e-04
seob4191	4C	2.69e-04
mioc2726	4C	2.7e-04

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fcrb6012	4C	3.0e-04	fcrc3750	4C	6.22e-04
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fcrc0112	4C	3.06e-04	mioc7686	4C	6.41e-04
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mioa1585	4C	3.13e-04	fcrc3009	4C	6.69e-04
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seoa5662	4C	3.21e-04	mioa6418	4C	7.09e-04
seoa0486	4C	3.23e-04	mioc0950	4C	7.19e-04
seoa2949	4C	3.29e-04	miod1925	4C	7.23e-04
fcrc0487	4C	3.31e-04	ncrc9910	4C	7.24e-04
ncr3837	4C	3.33e-04	seoa0221	4C	7.25e-04
mioa7317	4C	3.37e-04	mioc5532	4C	7.27e-04
hfcr1863	4C	3.39e-04	mioc4089	4C	7.46e-04
fcr257 3	4C	3.43e-04	mioc7764	4C	7.57e-04
seob5743	4C	3.48e-04	seoa4681	4C	7.58e-04
mioa5695	4C	3.5e-04	seob6131	4C	7.61e-04
fcrb6968	4C	3.6e-04	seob3303	4C	7.67e-04
seob7584	4C	3.63e-04	mioa4552	4C	7.78e-04
ncrc0342	4C	3.76e-04	ncr8843	4C	7.83e-04
ncrc5417	4C	3.79e-04	seoa9537	4C	7.83e-04
seob3854	4C	3.91e-04	seob9574	4C	7.88e-04
seob6279	4C	3.96e-04	seob0308	4C	7.98e-04
fcrb8504	4C	3.99e-04	mioa1193	4C	8.04e-04
seob3367	4C	4.04e-04	seob1318	4C	8.04e-04
fcrb7830	4C	4.09e-04	hfcr6687	AC	8.17e-04
seob3182	4C	4.09e-04	fcrb1496	4C	8.19e-04
hfcr4489	4C	4.15e-04	miob8320	4C	8.21e-04
fcrb4428	4C	4.19e-04	seob0376	AC	8.21e-04
ncr0898	4C	4.21e-04	seoa8979	4C	8.23e-04
mioc8057	4C	4.39e-04	seoa9357	4C	8.24e-04
miobl231	4C	4.43e-04	ncrb0782	4C	8.27e-04
mioc2911	4C	4.49e-04	seob3923	4C	8.31e-04
fcr5559	4C	4.69e-04	ncr2293	4C	8.33e-04
seoa6497	4C	4.69e-04	seob0755	AC	8.35e-04
seoa6598	4C	4.73e-04	hfcr0478	4C	8.43e-04
fcrl463	4C	4.77e-04	ncr0733	4C	8.46e-04
fcr2103	4C	4.84e-04	hfcr5220	4C	8.48e-04
seob3191	4C	4.89e-04	ncrc5844	4C	8.48e-04
seobl319	4C	4.91e-04	mioa5355	4C	8.58e-04
miod6845	4C	4.99e-04	ncr8041	4C	8.61e-04
ncrc5663	4C	5.0e-04	fcr0824	4C	8.62e-04
seob6844	4C	5.04e-04	seob2994	4C	8.67e-04
mioa9510	4C	5.12e-04	seob3119	AC	8.73e-04
ncrc4597	4C	5.18e-04	fcrb8668	4C	8.75e-04
fcr3664	4C	5.26e-04	seob6206	4C	8.88e-04
seob4 669	4C	5.33e-04	mioa6731	4C	8.9e-04
seob9282	4C	5.33e-04	fcrb2556	4C	8.98e-04
ncr7813	4C	5.48e-04	ncr5568	4C	9.0e-04
seoa7530	4C	5.56e-04	mioc4667	4C	9.01e-04
seob0248	4C	5.56e-04	mioa5696	4C	9.03e-04
seoa0288	4C	5.7e-04	hfcr1163	4C	9.1e-04
mioa6064	4C	5.71e-04	miob7156	4C	9.1e-04
seoc6703	4C	5.75e-04	ncr0808	4C	9.12e-04
miob9907	4C	5.82e-04	hfcr3019	4C	9.19e-04
ncrc8903	4C	5.88e-04	fcrc3048	4C	9.26e-04
seob2148	4C	5.88e-04	fcrb9454	4C	9.3e-04

Se'oa'631'5-	4C	9.37e-04	miod1195	4C	1.431152e-03
fcrb9655	4C	9.37e-04	fcrb24 84	4C	1.447281e-03
ncrc0715	4C	9.39e-04	mioc6987	4C	1.45158e-03
seoa8348	4C	9.56e-04	fcrl879	4C	1.452287e-03
fcrb9420	4C	9.64e-04	mioa6721	4C	1.457877e-03
seob6272	4C	9.74e-04	seob6486	4C	1.458125e-03
fcr2798	4C	9.78e-04	seoa3516	4C	1.46015e-03
seoc1996	4C	9.81e-04	ncr0025	4C	1.469905e-03
hfcr6486	4C	9.83e-04	hfcr1310	4C	1.478083e-03
ncrc9280	4C	1.0e-03	fcr3101	4C	1.487316e-03
seoa5090	4C	1.007122e-03	fcrb9959	4C	1.48762e-03
mioa2173	4C	1.00875e-03	fcrb5214	4C	1.490057e-03
ncrb5595	4C	1.013841e-03	ncrb4182	4C	1.50404e-03
fcrb2933	4C	1.016356e-03	ncrb3702	4C	1.511682e-03
fcr7043	4C	1.016688e-03	ncr7595	4C	1.511845e-03
miod1236	4C	1.028284e-03	mioa0577	4C	1.518616e-03
seob64 4 6	4C	1.030592e-03	ncr8975	4C	1.524283e-03
seob2936	4C	1.039004e-03	seob4579	4C	1.527065e-03
ncrb8693	4C	1.046851e-03	fcr4128	4C	1.53383e-03
mioa9154	4C	1.070876e-03	ncrc5162	4C	1.553082e-03
seoa6658	4C	1.070876e-03	ncr5168	AC	1.55656e-03
ncrb2288	AC	1.076406e-03	ncrb3001	4C	1.557302e-03
fcrc7388	4C	1.081745e-03	fcrb8187	4C	1.573331e-03
fcrb9588	4C	1.089146e-03	ncrb4039	4C	1.584396e-03
miob8572	4C	1.097548e-03	miodl 811	4C	1.590472e-03
seob8204	4C	1.099016e-03	seoa9740	4C	1.598061e-03
miob3982	4C	1.105438e-03	mioa3080	4C	1.603678e-03
ncrc0174	4C	1.123083e-03	hfcr3500	4C	1.610297e-03
miod5310	4C	1.129688e-03	mioa8852	4C	1.618546e-03
hfcr5604	4C	1.140826e-03	mioa0494	4C	1.619093e-03
seoa7443	4C	1.140826e-03	seoa0111	4C	1.622252e-03
fcrbl855	4C	1.142061e-03	ncr3782	AC	1.63287e-03
ncr3713	4C	1.150048e-03	ncrc5569	AC	1.637974e-03
fcrb9269	4C	1.150659e-03	mioc6075	4C	1.640004e-03
mioa3395	4C	1.154329e-03	seoa111 8	4C	1.653668e-03
seob8368	4C	1.154981e-03	mioa4076	4C	1.665908e-03
seob0122	4C	1.171391e-03	seoa4305	4C	1.678116e-03
mioa0535	4C	1.173792e-03	fcrc6826	4C	1.682437e-03
miob1062	4C	1.177608e-03	mioa1015	4C	1.68644e-03
seob1793	4C	1.177608e-03	seob2185	4C	1.70771e-03
seoc1593	4C	1.178329e-03	fcrc2090	4C	1.719751e-03
miob9336	4C	1.178419e-03	seoa9870	4C	1.728369e-03
ncrc0090	4C	1.185916e-03	seob1414	4C	1.747109e-03
seob1862	4C	1.201035e-03	seoa9828	4C	1.753504e-03
seoa3105	4C	1.211669e-03	fcrb3165	4C	1.760242e-03
seob3670	4C	1.23222e-03	seoa1598	4C	1.770719e-03
hfcr6406	4C	1.239885e-03	fcrc2099	4C	1.790284e-03
miob4867	4C	1.2560936e-03	seob1766	4C	1.794891e-03
ncrc1140	4C	1.256093e-03	hfcr1743	AC	1.798381e-03
fcr5720	4C	1.2614e-03	seob1908	AC	1.798381e-03
miod6238	4C	1.278208e-03	seob4545	4C	1.798381e-03
mioc7331	4C	1.301405e-03	fcrc5516	4C	1.799844e-03
ncrc6005	4C	1.305117e-03	hfcr5228	4C	1.800664e-03
miod1942	4C	1.305311e-03	mioa5692	4C	1.800664e-03
mioa0909	4C	1.312146e-03	seob4555	AC	1.800664e-03
seob1879	4C	1.347303e-03	fcrbl420	4C	1.801622e-03
miob6904	4C	1.355302e-03	fcr3595	4C	1.803204e-03
mioc2880	4C	1.356947e-03	ncr2666	4C	1.803204e-03
seob6368	4C	1.361315e-03	ncr2905	4C	1.815247e-03
ncr2472	4C	1.365497e-03	fcrb8940	4C	1.834572e-03
ncrb8385	4C	1.369106e-03	miob54 95	4C	1.838333e-03
fcrl756	4C	1.392986e-03	ncr3368	4C	1.838333e-03
fcr2088	4C	1.408465e-03	fcrbl807	4C	1.859263e-03
miob3348	4C	1.428182e-03	ncrc5054	4C	1.860924e-03

seob06525	4C	r:861275e-03	seoa5577	4C	2.367648e-03
seoc0924	4C	1.861444e-03	seoa5235	4C	2.369839e-03
seoa8399	4C	1.88316e-03	ncrb8273	4C	2.393454e-03
fcrb81β1	4C	1.885852e-03	fcrb3134	4C	2.402582e-03
mioal520	AC	1.887743e-03	mioa9294	4C	2.412283e-03
fcrb8740	4C	1.892065e-03	hfcr1697	4C	2.435634e-03
seob2810	4C	1.901388e-03	ncrc5369	4C	2.435634e-03
fcr5758	4C	1.909224e-03	seob5219	4C	2.439695e-03
mioc8423	4C	1.916815e-03	mioc6114	4C	2.46067e-03
ncrc9729	4C	1.919544e-03	miob8143	4C	2.489145e-03
hfcr5695	4C	1.956159e-03	seoall73	4C	2.502414e-03
seoc4380	4C	1.956915e-03	seoa3670	4C	2.543649e-03
fcrb2871	4C	1.964974e-03	miob0189	4C	2.57178e-03
miod0592	4C	1.985622e-03	miob2705	4C	2.574869e-03
ncrc0150	4C	1.985831e-03	seoa8232	4C	2.578028e-03
fcrb2060	4C	1.99746e-03	fcr5123	4C	2.597097e-03
hfcr0415	4C	1.99746e-03	inioa6854	4C	2.602922e-03
seoa7555	4C	1.99746e-03	ncrc5061	4C	2.618499e-03
fcr2079	4C	2.016524e-03	seoc0369	4C	2.638551e-03
miod0340	4C	2.021229e-03	fcr0139	4C	2.651872e-03
seoc1025	4C	2.02575e-03	fcr7004	AC	2.657357e-03
fcrb8080	4C	2.025786e-03	mioa9505	4C	2.658032e-03
mioc0824	4C	2.034483e-03	mioc8434	4C	2.661058e-03
seob0201	4C	2.050861e-03	seob7465	4C	2.711845e-03
fcrb9720	4C	2.057033e-03	fcrb4409	4C	2.722505e-03
fcr5470	4C	2.060807e-03	seoc0276	4C	2.725532e-03
ncrc4079	4C	2.066895e-03	fcr0999	4C	2.730498e-03
hfer0624	4C	2.085152e-03	hfcr4488	4C	2.731117e-03
ncrb3329	4C	2.085152e-03	seoa0563	4C	2.731117e-03
seob6015	4C	2.09993e-03	fcr3559	4C	2.734761e-03
fcr4782	4C	2.115262e-03	mioal660	4C	2.749542e-03
fcrbl733	4C	2.120875e-03	seoc0513	4C	2.753054e-03
fcrb9843	4C	2.120966e-03	ncrc9712	4C	2.760324e-03
ncr0238	4C	2.1386e-03	miod5707	4C	2.770552e-03
mioc7895	4C	2.143605e-03	miob8711	4C	2.809832e-03
fcrb3718	4C	2.145161e-03	seob3307	4C	2.811695e-03
seob6670	4C	2.155534e-03	seob9946	4C	2.84126e-03
seob8501	4C	2.166556e-03	mioc7744	4C	2.845792e-03
miod2525	4C	2.174809e-03	seoa8543	4C	2.874349e-03
mioc6312	4C	2.199817e-03	miob3898	4C	2.881671e-03
hfcr1733	4C	2.209362e-03	seoa8642	4C	2.881671e-03
ncrb4957	4C	2.210616e-03	mioa7140	4C	2.898369e-03
seob0089	4C	2.211811e-03	seoa7546	4C	2.919393e-03
hfcr6651	4C	2.216864e-03	fcr4763	4C	2.928101e-03
hfcr1302	4C	2.226095e-03	miob5016	4C	2.928675e-03
seob4050	4C	2.226095e-03	fcrcl849	4C	2.952953e-03
fcrb2536	4C	2.230106e-03	ncrc3464	4C	2.965232e-03
mioa8851	4C	2.23281e-03	fcrb3483	4C	2.983789e-03
fcr3121	4C	2.234303e-03	seob9649	AC	2.997767e-03
seoa2012	4C	2.255872e-03	seoa4600	AC	2.998627e-03
seoc1118	4C	2.258564e-03	seoa0008	AC	3.007551e-03
fcr7419	4C	2.260166e-03	seob0168	4C	3.016761e-03
seob7432	4C	2.272415e-03	seob2221	4C	3.044324e-03
mioc4318	4C	2.279552e-03	ncrc5079	AC	3.050155e-03
fcr0695	4C	2.279562e-03	fcrb9481	AC	3.074875e-03
seoa8443	4C	2.285067e-03	ncrb0054	AC	3.127244e-03
fcr06566	4C	2.298537e-03	seoa3533	4C	3.13784e-03
miobl326	4C	2.329343e-03	miod5369	4C	3.159305e-03
miob4475	4C	2.330183e-03	seoa5683	4C	3.160612e-03
ncrc9469	4C	2.33257e-03	fcrb2218	4C	3.164316e-03
fcr0224	4C	2.334725e-03	mioa0407	4C	3.167049e-03
miod6467	4C	2.34447e-03	fcrb7693	4C	3.203691e-03
seob4273	4C	2.355513e-03	seoa9160	4C	3.207335e-03
hfcr6700	4C	2.362015e-03	miob4836	4C	3.218792e-03

rtct0'4's β'	4C	3.245 '826e-03
ncrb6530	4C	3.252247e-03
ncrc0100	4C	3.298912e-03
ncrc2776	4C	3.322303e-03
ncrb3942	4C	3.332792e-03
ncrb0513	4C	3.349517e-03
ncrl387	4C	3.356296e-03
ncrb0145	4C	3.359507e-03
fcrb3017	4C	3.376066e-03
ncrc5091	4C	3.385978e-03
fcrc0241	4C	3.422538e-03
seob0514	4C	3.423122e-03
ncr8866	4C	3.437719e-03
fcrc6888	4C	3.449333e-03
miob9065	4C	3.456747e-03
seoa0549	4C	3.456908e-03
mioa3514	4C	3.46245e-03
ncr3237	4C	3.46245e-03
ncrc4991	4C	3.4643e-03
seob3499	4C	3.4643e-03
ncrc9855	4C	3.46879e-03
seoa1036	4C	3.477733e-03
miod6029	4C	3.517177e-03
ncr2013	4C	3.51941e-03
seob4972	4C	3.531512e-03
hfcr0130	4C	3.533831e-03
fcrb4378	4C	3.540302e-03
mioc3726	4C	3.550472e-03
seob2188	4C	3.584274e-03
seobl667	4C	3.640309e-03
seoa6661	4C	3.644681e-03
seoa1720	4C	3.649977e-03
seob2108	4C	3.666209e-03
ncr0478	4C	3.666381e-03
fcrb2249	4C	3.695741e-03
ncrb8134	4C	3.708615e-03
seob9750	4C	3.708927e-03
fcr6577	4C	3.735652e-03
hfcr0676	4C	3.735778e-03
miob24 92	4C	3.735778e-03
mioa1971	4C	3.738256e-03
seob5954	4C	3.738256e-03
miob8 932	4C	3.752087e-03
ncrc4808	4C	3.775334e-03
mioa5085	4C	3.775842e-03
seoa4586	4C	3.780119e-03
seoa3408	4C	3.790116e-03
hfcr0370	4C	3.8121e-03
mioa8774	4C	3.812303e-03
seobl187	4C	3.817075e-03
fcr3155	4C	3.832262e-03
ncr2182	4C	3.847974e-03
ncrc9557	4C	3.849741e-03
miob0974	4C	3.874121e-03
fcrb6009	4C	3.925377e-03
mioc5643	4C	3.926872e-03
miod7243	4C	3.934025e-03
ncrc3434	4C	3.939813e-03
seob0200	4C	3.979023e-03
hfcr9613	4C	4.011004e-03
miob4055	4C	4.011569e-03
miob4956	4C	4.05244e-03
miob0154	4C	4.08718e-03
seoa2442	4C	4.093483e-03

seob3869	4C	4.093483e-03
seoa2641	4C	4.10684e-03
seoa6620	4C	4.118774e-03
mioc0741	4C	4.154883e-03
fcrb9694	4C	4.20251e-03
ncrc4531	4C	4.208322e-03
ncrb7292	4C	4.220365e-03
miod4140	4C	4.240281e-03
hfcr0045	4C	4.261381e-03
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seoc2191	4C	4.272909e-03
mioc3716	4C	4.281847e-03
fcrb3725	4C	4.312352e-03
seoa9711	4C	4.329889e-03
ncrc9772	4C	4.399985e-03
seoa6573	4C	4.452642e-03
mioa0380	4C	4.489956e-03
mioa1097	4C	4.509349e-03
seob3462	4C	4.509349e-03
mioa8811	4C	4.52055e-03
fcrc0959	4C	4.530274e-03
seob3154	4C	4.531125e-03
mioa0890	4C	4.570501e-03
fcrc6335	4C	4.58749e-03
ncrc5760	4C	4.601184e-03
mioa1354	4C	4.601629e-03
mioa1427	4C	4.633362e-03
ncrc3840	4C	4.633362e-03
seoa0799	4C	4.633362e-03
seoa4040	4C	4.639931e-03
miod4348	4C	4.655192e-03
seoa7902	4C	4.66728e-03
seoa2978	4C	4.686163e-03
ncrc5150	4C	4.691321e-03
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fcrc7047	4C	4.744327e-03
seob5562	4C	4.746427e-03
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mioa6739	4C	4.778158e-03
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hfcr6468	4C	4.79651e-03
ncrb8253	4C	4.799003e-03
seoa5986	4C	4.799003e-03
miob5736	4C	4.80808e-03
seoa7517	4C	4.870405e-03
mioa0826	4C	4.884238e-03
fcrb4415	4C	4.898061e-03
mioa6621	4C	4.904983e-03
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ncrc6795	4C	4.933324e-03
seoa3847	4C	4.942903e-03
mioa9831	4C	4.946737e-03
fcr5625	4C	4.99519e-03
ncr3684	4C	5.017654e-03
hfcr2287	4C	5.054567e-03
fcrc2576	4C	5.063792e-03
fcrc0430	4C	5.084925e-03
ncrc5672	4C	5.11611e-03
fcr6497	4C	5.124127e-03
mioa5468	4C	5.124127e-03
seoa6923	4C	5.124127e-03
miob0865	4C	5.128184e-03
mioa8946	4C	5.185969e-03

fct0 G'g"	4C"	5.197151e-03	ncr4550	4C	6.099859e-03
ncrc5039	4C	5.197151e-03	mioc7444	4C	6.13308e-03
hfcr5009	4C	5.200779e-03	seoa3704	4C	6.146625e-03
mioc2639	4C	5.218734e-03	hfcr1265	4C	6.161536e-03
fcrc4360	4C	5.250167e-03	seob0321	4C	6.178138e-03
fcrb4241	4C	5.25156e-03	fcrb7981	4C	6.206858e-03
fcrc6563	4C	5.282794e-03	seob3520	4C	6.215767e-03
seoa2122	4C	5.287317e-03	ncrc0101	4C	6.221193e-03
fcro768	4C	5.301658e-03	ncrb6833	AC	6.229488e-03
mioc3854	4C	5.307923e-03	ncr0075	AC	6.26855e-03
fcrc1745	4C	5.32716e-03	mioa8952	4C	6.270044e-03
mioa4196	4C	5.344108e-03	ncr0766	4C	6.276933e-03
mioc8619	4C	5.353299e-03	ncrc6817	4C	6.276933e-03
ncrc4654	4C	5.369673e-03	mioa2013	4C	6.287512e-03
seob4515	4C	5.369673e-03	seob9485	AC	6.301111e-03
ncr6232	4C	5.407943e-03	mioc4142	AC	6.383134e-03
mioc2074	4C	5.414445e-03	miob4593	4C	6.402894e-03
ncrc9758	4C	5.427537e-03	fcrc5779	4C	6.474618e-03
ncr4545	4C	5.438486e-03	seoa6197	4C	6.486591e-03
ncr9429	4C	5.458161e-03	seob0703	4C	6.50097e-03
fcrb8110	4C	5.478029e-03	miob6821	4C	6.505915e-03
seoa0029	4C	5.480759e-03	ncrb8343	4C	6.552284e-03
seobOCm	4C	5.509288e-03	seoa5911	4C	6.597064e-03
mioc0222	4C	5.512684e-03	mioc2166	4C	6.609859e-03
seoa1856	4C	5.531453e-03	fcrb9352	4C	6.647947e-03
fcrc6854	4C	5.540234e-03	fcrb2292	AC	6.653984e-03
fcrc2082	4C	5.550695e-03	seob4039	AC	6.713668e-03
seob2987	4C	5.569207e-03	fcrb5422	4C	6.7264e-03
mioa1473	4C	5.580693e-03	ncrc2119	4C	6.756046e-03
fcrb9871	4C	5.592316e-03	seob9552	4C	6.762169e-03
seob4030	4C	5.594557e-03	fcrb3841	4C	6.780973e-03
mioa2158	4C	5.639619e-03	miob6988	4C	6.80749e-03
fcrc4795	4C	5.649808e-03	hfcr1760	4C	6.835569e-03
seob5726	4C	5.65605e-03	hfcr5473	4C	6.857537e-03
fcrb7852	4C	5.667225e-03	fcrc3415	4C	6.861942e-03
ncrl550	4C	5.713507e-03	hfcr5987	4C	6.867295e-03
mioa3471	4C	5.716154e-03	seoa0926	4C	6.867921e-03
mioa0132	4C	5.738039e-03	miobl115	AC	6.869801e-03
mioa8647	AC	5.768458e-03	fcrc4380	AC	6.880921e-03
mioa1445	4C	5.811166e-03	miob5412	4C	6.89987e-03
seoc3588	4C	5.826485e-03	ncr6335	4C	6.907692e-03
ncrc3468	4C	5.832748e-03	seoa3628	4C	6.949533e-03
miob9052	4C	5.833135e-03	seob9772	4C	6.956817e-03
mioc5664	4C	5.835444e-03	mioa9581	4C	6.958796e-03
fcrb2137	4C	5.84394e-03	ncr248 6	4C	6.982956e-03
hfcr5207	4C	5.84394e-03	miob0877	4C	6.983468e-03
mioa2327	4C	5.859459e-03	ncr0240	4C	6.999641e-03
fcrb9147	4C	5.861796e-03	seoa6137	4C	7.003723e-03
seob5458	4C	5.862878e-03	mioa1337	4C	7.012816e-03
seoa5554	4C	5.877316e-03	fcrc6415	4C	7.029261e-03
fcrb5254	4C	5.890258e-03	seobl385	4C	7.029563e-03
seoa1552	4C	5.896449e-03	ncrb8207	4C	7.041692e-03
seoa3230	4C	5.898784e-03	hfcr1914	4C	7.057729e-03
fcrl844	4C	5.913324e-03	ncrl351	4C	7.095309e-03
fcrb52 67	4C	5.942373e-03	seob2803	AC	7.108081e-03
fcrb6896	4C	5.949973e-03	seob5012	AC	7.108081e-03
seoa4524	4C	5.958281e-03	seob7474	4C	7.109226e-03
fcrc0094	4C	5.96467e-03	mioc7818	4C	7.166952e-03
miob3684	4C	5.971717e-03	mioa4241	4C	7.17269e-03
fcrc6390	4C	6.000719e-03	seoa2136	4C	7.196923e-03
seobl737	4C	6.007946e-03	seoa9935	4C	7.197022e-03
fcrc2074	4C	6.018802e-03	fcrc5339	4C	7.199684e-03
seoc2131	4C	6.08684e-03	miob2836	4C	7.210691e-03
fcrc0608	4C	6.096137e-03	mioa0104	4C	7.212787e-03

ncr3948	4C	7.237837e-03	miob2855	4C	8.459946e-03
mioa6093	4C	7.268139e-03	seoa1263	4C	8.459946e-03
seoa1559	4C	7.27224e-03	seoa7459	4C	8.497761e-03
hfc9549	4C	7.295285e-03	seob2658	4C	8.497761e-03
seobl191	4C	7.325599e-03	ncrc6871	4C	8.532381e-03
miob4058	4C	7.329008e-03	seoa7474	4C	8.568552e-03
fcr5723	4C	7.369143e-03	ncrc1502	AC	8.575665e-03
mioa0328	4C	7.377639e-03	fcrc2997	4C	8.587588e-03
seoc3993	4C	7.378389e-03	mioa9891	4C	8.689171e-03
miod5703	4C	7.424044e-03	hfc93928	4C	8.732239e-03
fcr6044	4C	7.452917e-03	mioa2478	4C	8.749534e-03
seob6560	4C	7.500236e-03	seoa7509	AC	8.755591e-03
fcrβ01β	4C	7.53305e-03	mioa6738	4C	8.769774e-03
miod6162	4C	7.584327e-03	seoa7178	4C	8.77984e-03
seoa2363	4C	7.617361e-03	seob5319	4C	8.811962e-03
seob2169	4C	7.634987e-03	fcrc0654	4C	8.812335e-03
fcr6616	4C	7.680441e-03	miobl506	AC	8.830271e-03
fcr6931	4C	7.680441e-03	miodl331	AC	8.912821e-03
seoa6152	4C	7.680441e-03	seoa5547	4C	8.932311e-03
fcr4622	4C	7.681578e-03	seob3169	4C	8.97374e-03
mioal324	4C	7.698039e-03	mioa3629	4C	8.97697e-03
fcrb7944	4C	7.767483e-03	miob3696	4C	8.981708e-03
ncrl802	4C	7.784697e-03	fcrc4054	4C	8.989376e-03
seob7729	AC	7.786058e-03	mioa2413	4C	9.00025e-03
seob2966	4C	7.806257e-03	fcr2160	4C	9.030333e-03
seob4108	4C	7.828518e-03	ncr4539	4C	9.030333e-03
ncr2484	4C	7.879438e-03	mioa0707	4C	9.037843e-03
ncrb3585	4C	7.879438e-03	ncr3496	4C	9.054107e-03
seoa0913	AC	7.91186e-03	ncrc0667	4C	9.054835e-03
ncr0612	4C	7.925456e-03	seob4807	AC	9.055692e-03
mioc7998	4C	7.939586e-03	ncrc9428	AC	9.100754e-03
seob9872	4C	7.94076e-03	mioa2652	AC	9.114417e-03
mioc0375	4C	7.952967e-03	miob34 61	4C	9.117224e-03
fcrb6768	AC	7.961352e-03	fcrb8877	AC	9.135346e-03
seoa3245	4C	7.995448e-03	fcr0707	AC	9.142522e-03
miob7309	4C	8.045055e-03	mioa9649	AC	9.154014e-03
hfc95905	4C	8.045743e-03	miob3938	AC	9.20224e-03
miob2227	4C	8.045772e-03	seoa5933	AC	9.204501e-03
fcrb7113	4C	8.05715e-03	miob5675	AC	9.254824e-03
hfc96501	4C	8.058052e-03	seoa2391	4C	9.263207e-03
ncrb1373	4C	8.093663e-03	fcrb6191	4C	9.290334e-03
fcrc0295	4C	8.130412e-03	ncrc0383	4C	9.294641e-03
mioa9717	4C	8.135586e-03	miob4743	AC	9.329707e-03
seob4209	4C	8.135586e-03	ncrb3284	AC	9.343469e-03
miod2665	4C	8.152135e-03	ncrc6953	4C	9.345199e-03
fcrc3358	4C	8.156756e-03	ncrc4757	4C	9.409517e-03
fcr4 965	4C	8.164022e-03	seob5032	4C	9.43118e-03
mioc6997	4C	8.185087e-03	ncrb6453	4C	9.583937e-03
miobl4 93	4C	8.197903e-03	ncrc5553	4C	9.583937e-03
fcr0593	4C	8.19812e-03	seob0418	4C	9.583937e-03
fcr4129	4C	8.215271e-03	seob0810	4C	9.583937e-03
ncr0912	4C	8.275646e-03	mioa4548	4C	9.611891e-03
seob0466	4C	8.285506e-03	fcrc5846	AC	9.622113e-03
mioa9630	4C	8.304775e-03	fcrbl503	AC	9.664889e-03
ncrb6192	4C	8.312148e-03	ncrc1873	AC	9.678381e-03
seoc2824	4C	8.3183e-03	ncrc0846	AC	9.679567e-03
seoa0070	4C	8.335344e-03	seob3064	4C	9.679567e-03
seoa1736	4C	8.342294e-03	seob5778	4C	9.689819e-03
miob9848	4C	8.369066e-03	fcrb5537	4C	9.69729e-03
seoa1104	4C	8.378822e-03	hfcrl419	4C	9.717051e-03
ncr0634	4C	8.421583e-03	mioa6035	4C	9.789009e-03
seoa5465	4C	8.437929e-03	seoa0003	4C	9.794242e-03
seob8065	4C	8.459463e-03	seoc4416	4C	9.841173e-03
			seob6492	4C	9.84478e-03

ncrb1802	4C	9.861386e-03	fcrb8162	4C	0.01
seob8817	4C	9.894482e-03	fcrb3702	4C	0.01
fcrb1578	AC	9.906745e-03	seoa4017	AC	0.01
fcz2164	AC	9.970513e-03	seob5441	AC	0.01
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miob8214	4C	0.01	mioa2536	4C	0.01
miod2837	4C	0.01	seoc3876	4C	0.01
mioc4366	4C	0.01	hfcr5237	4C	0.01
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fcr0955	AC	0.01	mioa9033	AC	0.01
fcrc3240	4C	0.01	fcr3823	AC	0.01
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ncr0132	AC	0.01	ncrb4390	AC	0.01
seoa0511	AC	0.01	hfcr1101	AC	0.01
fcrb2713	AC	0.01	fcrc0350	AC	0.01
miob6797	AC	0.01	fcrb4570	AC	0.01
ncrb1956	AC	0.01	hfcr0237	AC	0.01
fcrc5351	AC	0.01	hfcr6716	AC	0.01
mioa2580	AC	0.01	inioc1609	AC	0.01
fcrb3651	AC	0.01	ncrc0292	AC	0.01
hfcr0829	AC	0.01	mioc3906	AC	0.01
seob1574	AC	0.01	seob3007	AC	0.01
seoa8556	AC	0.01	miod6731	AC	0.01
mioa1285	AC	0.01	fcrb7588	AC	0.01
seoa2162	AC	0.01	seoc2226	AC	0.01
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miob4574	AC	0.01	ncrb4319	AC	0.01
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miob4037	AC	0.01	fcrc6990	AC	0.01
seoa9814	AC	0.01	seob5927	AC	0.01
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seob6836	AC	0.01	seoa7295	AC	0.01
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mioa7015	AC	0.01	seob9882	AC	0.01
miob2743	AC	0.01	ncr3434	AC	0.01
seoa8300	AC	0.01	miob2700	AC	0.01
fcrb6785	4C	0.01	seob3170	AC	0.01
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fcrb5527	4C	0.01	miob4673	AC	0.01
mioa5461	4C	0.01	ncrc3529	AC	0.01
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fcrb4109	4C	0.01	seob5213	AC	0.01
seoa4264	4C	0.01	seoa6344	AC	0.01
fcrc2948	4C	0.01	ncrc5806	AC	0.01
inioc0121	4C	0.01	seob0478	AC	0.01
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hfcr2148	4C	0.01	fcrb3704	AC	0.01
seob3887	4C	0.01	mioa5691	AC	0.01
ncrb6087	4C	0.01	ncr4202	AC	0.01
seob1916	4C	0.01	fcrc1216	AC	0.01
fcrc5577	4C	0.01	seob6133	AC	0.01
fcrb2635	4C	0.01	hfcr1671	AC	0.01
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seob4122	AC	0.01	fcrl633	4C	0.01
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			seoc3603	4C	0.01

-fcrb 6834"	4C	0.01	mioa8864	4C	0.01
fcr2958	4C	0.01	fcrb7453	4C	0.01
seoa2428	4C	0.01	seoa9729	4C	0.01
seoa1056	4C	0.01	ncrc6617	4C	0.01
miob04 96	4C	0.01	hfcr1795	4C	0.01
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seoc2447	4C	0.01	fcrb6432	4C	0.01
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mioa7323	4C	0.01	fcr6881	4C	0.01
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miod4407	4C	0.01	fcr0997	4C	0.01
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seob9645	4C	0.01	fcrb8119	4C	0.01
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mioa5097	4C	0.01	miob4793	4C	0.01
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mioc0899	4C	0.01	seoc7281	4C	0.01
fcr5509	4C	0.01	mioc7807	4C	0.01
ncrc4296	4C	0.01	mioc10 βo	4C	0.01
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fcr6937	4C	0.01	mioc7370	4C	0.01
miod3468	4C	0.01	seoa5429	4C	0.01
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fcrb5675	4C	0.01	seob4570	4C	0.01
ncrc3453	4C	0.01	fcr1557	4C	0.01
miob37 63	4C	0.01	ncrc9867	4C	0.01
seoa0457	4C	0.01	mioc2039	4C	0.01
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ncrc3045	4C	0.01	seoa1653	4C	0.01
fcr5176	4C	0.01	ncrc6382	4C	0.01
fcrb5536	4C	0.01	miob7638	4C	0.01
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mioa2421	4C	0.01	mioa364 6	4C	0.01
ncrl235	4C	0.01	hfcr2314	4C	0.01
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'fict 7Cr85-	'ifc	'G.o'i'	seob9480	4C	O .01
fcrb3244	4C	0.01	seob4766	4C	O .01
seoa0931	4C	0.01	ncrb6661	4C	O .01
miob3471	4C	0.01	seob9756	4C	O .01
fcrb84 65	4C	0.01	fcrb6829	4C	O .01
seoc2253	4C	0.01	miod.6947	4C	O .01
ncr7267	4C	0.01	fcr1833	4C	O .01
fcrb50 92	4C	0.01	fcr3983	4C	O .01
ncrb4339	4C	0.01	mioal531	4C	O .01
mioc7561	4C	0.01	mioa8796	4C	O .01
mioa9666	4C	0.01	seoa6621	4C	O .01
ncrc1765	4C	0.01	hfcrc6680	4C	O .01
ncr8811	4C	0.01	fcr6386	4C	O .01
fcrc5797	4C	0.01	seoa3863	4C	O .01
mioal079	4C	0.01	fcr1305	4C	O .01
hfcrc3413	4C	0.01	fcrb8092	4C	O .01
miob0973	4C	0.01	fcrb2596	4C	O .01
hfcrl709	4C	0.01	ncrc3141	4C	O .01
hfcrc0285	4C	0.01	seoa8239	4C	O .01
fcr6314	4C	0.01	fcrb11 60	4C	O .01
ncr0335	4C	0.01	ncrb6327	4C	O .01
fcr4927	AC	0.01	fcrc2969	4C	O .01
seoc0394	4C	0.01	ncrc6127	4C	O .01
seob4945	4C	0.01	mioa8539	4C	O .01
fcrb4739	4C	0.01	ncr8693	4C	O .01
fcrbl750	4C	0.01	fcr6266	4C	O .01
fcrc01 6β	4C	0.01	fcr4272	AC	O .01
seob2011	AC	0.01	fcrb242 6	AC	O .01
ncrc4089	4C	0.01	seob2077	AC	O .01
ncrc0803	4C	0.01	ncrc2859	4C	O .01
fcrc2007	4C	0.01	fcrbl763	4C	O .01
hfcrc2832	4C	0.01	mioa0152	AC	O .01
seoa3422	4C	0.01	seoc0866	AC	O .01
miob4157	4C	0.01	fcr0712	AC	O .01
ncr8893	4C	0.01	ncrb7102	AC	O .01
seoa3108	4C	0.01	hfcrc4423	AC	O .01
mioa0246	4C	0.01	ncrc2613	AC	O .01
miob7391	4C	0.01	seob7764	4C	O .01
mioa6091	AC	0.01	hfcrl641	4C	O .01
ncr3811	AC	0.01	miod0686	4C	O .01
seob5886	4C	0.01	fcrb9856	4C	O .01
mioc4641	4C	0.01	miobl789	4C	O .01
seob4539	4C	0.01	ncr6141	4C	O .01
mioc8379	4C	0.01	mioa9935	AC	O .01
seob8104	4C	0.01	seoa1979	AC	O .01
fcrb6251	4C	0.01	ncr7973	AC	O .01
fcrc0839	4C	0.01	seoa4727	AC	O .01
ncr8067	4C	0.01	fcrc5671	AC	O .01
seoa2272	4C	0.01	fcrb7392	AC	O .01
seoa0469	4C	0.01	seob6229	AC	O .01
ncr4118	4C	0.01	seoa2819	AC	O .01
seoa2817	4C	0.01	fcrb9639	AC	O .01
fcrb2020	4C	0.01	fcrb4294	AC	O .01
fcrb2305	4C	0.01	fcr0990	AC	O .01
ncrb8392	4C	0.01	mioa3799	AC	O .01
ncr7768	4C	0.01	miob0207	AC	O .01
hfcrc5150	4C	0.01	seoa3144	4C	O .01
seob7 658	4C	0.01	seob5490	AC	O .01
seobl322	4C	0.01	fcrb9680	AC	O .01
ncr9919	4C	0.01	fcr1855	AC	O .01
ncr3396	4C	0.01	seoa5742	AC	O .01
miob9185	4C	0.01	fcrc4045	AC	O .01
seoa9724	4C	0.01	seob2081	AC	O .01
ncrc1176	4C	0.01	fcr7042	AC	O .01

"M'b&l 4"40	4C	Tr"O'i			
ncrc3358	4C	0.01	seob0831	4C	0.02
ncrc2273	4C	0.01	ncrc9483	4C	0.02
ncrc5608	4C	0.01	seob5645	4C	0.02
fcr3323	4C	0.01	seocl264	4C	0.02
ncrb0262	4C	0.01	fcr2940	4C	0.02
seoa3639	4C	0.01	mioa2475	4C	0.02
mioc4534	4C	0.01	fcrcl660	4C	0.02
ncrc9023	4C	0.01	seob9067	4C	0.02
miob4238	4C	0.01	ncr5713	4C	0.02
ncr0615	4C	0.01	seob0745	4C	0.02
seob7744	4C	0.01	seoa4023	4C	0.02
ncrc3072	4C	0.01	seob8807	4C	0.02
fcrb3298	4C	0.01	fcrbl529	4C	0.02
mioa2156	4C	0.01	seoa3251	4C	0.02
fcrcl6174	4C	0.01	ncrc5363	4C	0.02
ncrc1952	4C	0.01	seob0763	4C	0.02
seoa5586	4C	0.02	fcrb4238	4C	0.02
seoa2962	4C	0.02	miod2330	4C	0.02
hfcrc0383	4C	0.02	miod0441	4C	0.02
miod5698	4C	0.02	hfcrc3441	4C	0.02
hfcrc3592	4C	0.02	seocl539	4C	0.02
fcrbl381	4C	0.02	miob3396	4C	0.02
seobl617	4C	0.02	mioc2539	4C	0.02
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mioc4925	4C	0.02	miob6372	4C	0.02
seobl243	4C	0.02	fcrb2591	4C	0.02
ncrb6037	4C	0.02	seob2807	4C	0.02
seob3533	4C	0.02	fcrcl4390	4C	0.02
fcrbl550	4C	0.02	hfcrc3404	4C	0.02
miod1448	4C	0.02	fcrb2308	4C	0.02
miob4712	4C	0.02	fcrb2160	4C	0.02
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fcrb2658	4C	0.02	miob304 4	4C	0.02
ncrc4885	4C	0.02	fcrcl763	4C	0.02
seoa7935	4C	0.02	seoa6887	4C	0.02
seoc4052	4C	0.02	ncrc7043	4C	0.02
seoa0256	4C	0.02	ncrc3276	4C	0.02
seoa0219	4C	0.02	miob4975	4C	0.02
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mioc4766	4C	0.02	fcrb2690	4C	0.02
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mioal055	4C	0.02	hfcrc2598	4C	0.02
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miob2687	4C	0.02	fcr5204	4C	0.02
ncr4416	4C	0.02	seob0253	4C	0.02
fcrb5204	4C	0.02	mioc4290	4C	0.02
ncrb8063	4C	0.02	fcr5618	4C	0.02
miob7109	4C	0.02	seoa3555	4C	0.02
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seobl834	4C	0.02	ncrc6981	4C	0.02
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fcrb6062	4C	0.02	hfcrc5181	4C	0.02
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seoa7608	4C	0.02	seocl664	4C	0.02
miob8 657	4C	0.02	fcrb9499	4C	0.02
fcr2276	4C	0.02	seob4612	4C	0.02
hfcrc0225	4C	0.02	seob7622	4C	0.02
			seoa8486	4C	0.02

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 fcr5316 4C 0.02

"- fdtbmri' w' :4c 'c '0 102 -"

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ncrc2888	4C	O.02	fcrc2678	4C	0.03
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seoa1089	4C	O.02	hfcrc0476	AC	0.03
mioa5531	4C	O.02	seob2134	AC	0.03
fcrc1947	4C	O.02	seob7404	AC	0.03
ncrb8751	4C	O.02	fcrcb8949	4C	0.03
miob6456	4C	O.02	ncrc4903	4C	0.03
ncrc2472	AC	O.02	seob2750	AC	0.03
seoa0949	4C	O.02	fcrcb5928	AC	0.03
fcrcb8014	4C	O.02	ncr3465	AC	0.03
seob2816	4C	O.02	seoc2173	4C	0.03
seoa8640	4C	O.02	mioc4420	4C	0.03
ncrb8437	4C	O.02	seob7946	4C	0.03
miod6560	4C	O.02	mioc2950	4C	0.03
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fcrcb1720	4C	O.02	mioa08 62	AC	0.03
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fcrc5160	4C	O.02	ncrb8 649	4C	0.03
fcrcb4275	4C	O.02	ncrb8821	4C	0.03
mioa4738	4C	O.02	fcrcb64 60	4C	0.03
ncrc0583	4C	O.02	fcrcb7616	4C	0.03
ncr5488	4C	O.02	ncr8995	4C	0.03
fcrc0591	4C	O.02	fcrcb3306	4C	0.03
mioa1494	4C	O.02	fcrcb4599	4C	0.03
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fcrc6592	4C	O.02	seoa7442	AC	0.03
seob4835	4C	O.02	ncr4551	AC	0.03
fcrcb7951	4C	O.03	seoa9930	AC	0.03
mioc2019	4C	O.03	hfcrc2820	4C	0.03
fcrcb2715	4C	O.03	mioc0317	AC	0.03
seoa7223	4C	O.03	seobl770	AC	0.03
seob2959	4C	O.03	seob3468	AC	0.03
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seob4480	4C	O.03	seoa9889	AC	0.03
raioa0308	4C	O.03	seoa7587	AC	0.03
ncr8542	4C	O.03	mioa22 68	4C	0.03
fcrcb6185	4C	O.03	fcrcb6102	AC	0.03
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seob3076	4C	O.03	seob0298	AC	0.03
fcrcb6747	4C	O.03	ncrb2544	AC	0.03
fcrcb2040	4C	O.03	fcrcb5416	AC	0.03
hfcrc2789	4C	O.03	fcrcb2198	AC	0.03
ncrl526	4C	O.03	miod5030	AC	0.03
ncrc1999	4C	O.03	seoa7249	AC	0.03
fcrcb4 696	4C	O.03	ncrc4047	AC	0.03
ncrc8881	4C	O.03	seob8311	AC	0.03
fcrcb2353	4C	O.03	ncr097 6	AC	0.03
mioa6174	4C	O.03	mioa8548	AC	0.03
seoa9389	4C	O.03	fcrc5222	AC	0.03
seoa2777	4C	O.03	seoa3910	AC	0.03
seob6558	4C	O.03	ncrc0793	AC	0.03
seoa2381	4C	O.03	mioc8694	AC	0.03
			fcrcb3169	AC	0.03

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 fcrb5177 AC 0.04
 seobl145 AC 0.04
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 ncr7852 AC 0.04

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ncrc5758 4C 0.04
fcr2018 4C 0.04
seoa3489 4C 0.04
hfcrl2516 4C 0.04
cr0682 4C 0.04
miob6595 4C 0.04
seoa9131 4C 0.04
ncr0547 4C 0.04
fcr0776 4C 0.04
fcrb4333 4C 0.04
hfcr1304 4C 0.04
fcr5006 4C 0.04
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miod1108 4C 0.04
mioa9007 4C 0.04
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hfcr1202 4C 0.04
ncrc5296 4C 0.04
seob3734 4C 0.04
ncr0531 4C 0.04
ncrc8976 4C 0.04
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seoc1159 4C 0.04
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seob0497 4C 0.04
hfcr0675 4C 0.04
miob3443 4C 0.04
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seob9543 4C 0.04
fcr4832 4C 0.04
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mioc3618 4C 0.04
seob6582 4C 0.04
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iniob9121 4C 0.04
fcr4 948 4C 0.04
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seoa6697 4C 0.04

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seob4419 4E 4.27e-06
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Sebas 23s

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miob3308	41	1.566301e-03	fcr4782	41	2.115262e-03
fcrb8187	41	1.573331e-03	fcrbl733	41	2.120875e-03
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miodl811	41	1.590472e-03	mioc7895	41	2.143605e-03
hfcrc3500	41	1.610297e-03	fcrb3718	41	2.145161e-03
mioa8852	41	1.618546e-03	seob6670	41	2.155534e-03
mioa0494	41	1.619093e-03	seob8501	41	2.166556e-03
seoa0111	41	1.622252e-03	miod2525	41	2.174809e-03
ncr3782	41	1.63287e-03	fcrb6187	41	2.177878e-03
ncrc5569	41	1.637974e-03	mioc6312	41	2.199817e-03
mioc6075	41	1.640004e-03	hfcrl733	41	2.209362e-03
mioa4076	41	1.665908e-03	ncrb4957	41	2.210616e-03
seoa4305	41	1.678116e-03	seob0089	41	2.211811e-03
seob2185	41	1.70771e-03	hfcrc6651	41	2.216864e-03
fcrc2090	41	1.719751e-03	hfcrl302	41	2.226095e-03
seoa9870	41	1.728369e-03	seob4050	41	2.226095e-03
seobl414	41	1.747109e-03	fcrb2536	41	2.230106e-03
seoa9828	41	1.753504e-03	mioa8851	41	2.23281e-03
fcrb3165	41	1.760242e-03	seoa2012	41	2.255872e-03
fcrc2099	41	1.790284e-03	seoc1118	41	2.258564e-03
seobl766	41	1.794891e-03	fcr7419	41	2.260166e-03
hfcrl743	41	1.798381e-03	seob7432	41	2.272415e-03
seobl908	41	1.798381e-03	mioc4318	41	2.279552e-03
seob4545	41	1.798381e-03	fcrc0695	41	2.279562e-03
fcrc5516	41	1.799844e-03	fcr3121	41	2.285067e-03
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mioa5692	41	1.800664e-03	seoa1065	41	2.312579e-03
seob4555	41	1.800664e-03	miob4475	41	2.330183e-03
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fcrb8161	41	1.885852e-03	seob5219	41	2.439695e-03
mioa1520	41	1.887743e-03	seoa5444	41	2.479655e-03
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seob2810	41	1.901388e-03	miob0189	41	2.57178e-03
fcr5758	41	1.909224e-03	miob2705	41	2.574869e-03
mioc8423	41	1.916815e-03	seoa8232	41	2.578028e-03
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seob4560	41	2.021887e-03	fcr0999	41	2.730498e-03
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miob8711	41	2.809832e-03	seoa6661	41	3.644681e-03
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seoa9814	41	2.836239e-03	seob2108	41	3.666209e-03
seob9946	41	2.84126e-03	ncr0478	41	3.666381e-03
mioc7744	41	2.845792e-03	fcrb2249	41	3.695741e-03
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miob3898	41	2.881671e-03	ncrb8134	41	3.708615e-03
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seoa0008	41	3.007551e-03	seoa3408	41	3.790116e-03
seob0168	41	3.016761e-03	mioa8774	41	3.812303e-03
seob2221	41	3.044324e-03	seoa7546	41	3.830009e-03
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seoa9160	41	3.207335e-03	hfcr9613	41	4.011004e-03
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ncrc2776	41	3.322303e-03	fcrb9694	41	4.20251e-03
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ncrb0513	41	3.349517e-03	miod4140	41	4.240281e-03
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fcrb3017	41	3.376066e-03	ncrc9877	41	4.26595e-03
ncrc5091	41	3.385978e-03	seoc2191	41	4.272909e-03
miob8274	41	3.404337e-03	mioc0567	41	4.279318e-03
fcr0241	41	3.422538e-03	mioc3716	41	4.281847e-03
seob0514	41	3.423122e-03	fcrb3725	41	4.312352e-03
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seob2797	41	3.6022e-03	seob4621	41	4.707547e-03
seob1667	41	3.640309e-03	fcr07047	41	4.744327e-03

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hfcrc6468	41	4.79651e-03	seoc3588	41	5.826485e-03
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fcr4795	41	5.649808e-03	miob6988	41	6.80749e-03
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seob9772	41	6.956817e-03	seob0466	41	8.285506e-03
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mioa7522	7H	0.04
mioa9062	7H	0.04
ncrl122	7H	0.04
ncr2288	7H	0.04
ncr3588	7H	0.04
ncr4040	7H	0.04
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ncr8413	7H	0.04
ncr9587	7H	0.04
ncrb74 65	7H	0.04
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seoa1776	7H	0.04
seoa3287	7H	0.04
seoa6466	7H	0.04
seoa6637	7H	0.04
seoa6677	7H	0.04
seoa6754	7H	0.04
seob0085	7H	0.04
seob0755	7H	0.04
seob2067	7H	0.04
seob4333	7H	0.04
seob5336	7H	0.04
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ncrc4759	71	2.552738e-03
ncrb0074	71	2.926149e-03
fcrb9565	71	3.208495e-03
fcrc2431	71	3.223683e-03
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ncrc3690	71	4.482529e-03
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seob0084	71	6.215714e-03
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seob4928	71	6.215714e-03
seoa6178	71	6.305954e-03
seob2658	71	7.212877e-03
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seob4029	71	8.879458e-03
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Table #8B

AffySpot	Tablet	pvalue
2017 94_s_at	IAA	1.68e-06
212640_at	IAA	4.74e-06
205060_at	IAA	4.18e-05
20468 β _at	IAA	6.09e-05
212648_at	IAA	6.57e-05
224 369_s_at	IAA	6.68e-05
221306_at	IAA	1.23e-04
22490 β _at	IAA	1.35e-04
203810_at	IAA	1.37e-04
20824 8_x_at	IAA	1.67e-04
221582_at	IAA	1.85e-04
216907_x_at	IAA	1.92e-04
211935_at	IAA	2.21e-04
212718_at	IAA	2.37e-04
20325 4_s_at	IAA	2.53e-04
215049_x_at	IAA	2.69e-04
202443_x_at	IAA	2.69e-04
208772_at	IAA	2.69e-04
21417 3_x_at	IAA	2.72e-04
230243_at	IAA	2.91e-04
201935_s_at	IAA	3.21e-04
224859_at	IAA	3.63e-04
206059_at	IAA	3.84e-04
204573_at	IAA	4.09e-04
218236_s_at	IAA	4.12e-04
200709_at	IAA	4.2e-04
202783_at	IAA	4.24e-04
203037_s_at	IAA	4.3e-04
206632_s_at	IAA	4.35e-04
1564785_at	IAA	4.37e-04
200697_at	IAA	4.56e-04
202638_s_at	IAA	4.65e-04
202786_at	IAA	4.73e-04
208704_x_at	IAA	4.78e-04
2128 63_x_at	IAA	4.91e-04
213906_at	IAA	4.93e-04
209585_s_at	IAA	5.43e-04
203392_s_at	IAA	5.55e-04
41577_at	IAA	6.09e-04
202471_s_at	IAA	6.18e-04
202 610_s_at	IAA	6.26e-04
200046_at	IAA	6.37e-04
200742_s_at	IAA	6.39e-04
204497_at	IAA	6.55e-04
204396_s_at	IAA	6.59e-04
203970_s_at	IAA	6.62e-04
201118_at	IAA	6.72e-04
201687_s_at	IAA	6.86e-04
209960_at	IAA	7.13e-04
212500_at	IAA	7.33e-04
2178 62_at	IAA	7.33e-04
217 970_s_at	IAA	7.43e-04
204806_x_at	IAA	7.43e-04
2032 64_s_at	IAA	7.48e-04
219183_s_at	IAA	7.53e-04
213291_s_at	IAA	7.54e-04
220999_s_at	IAA	7.75e-04
202096_s_at	IAA	7.86e-04
202720_at	IAA	7.88e-04
217197_x_at	IAA	8.02e-04

AffySpot	Tablet	pvalue
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219734_at	IAA	8.16e-04
91684_g_at	IAA	8.64e-04
213694_at	IAA	8.67e-04
200867_at	IAA	8.76e-04
202545_at	IAA	8.9e-04
207314_x_at	IAA	8.9e-04
211733_x_at	IAA	9.02e-04
202184^s_at	IAA	9.34e-04
214109_at	IAA	9.39e-04
214743_at	IAA	9.48e-04
221419_s_at	IAA	9.48e-04
211926_s_at	IAA	9.48e-04
211945_s_at	IAA	9.48e-04
200649_at	IAA	9.48e-04
216041_x_at	IAA	9.79e-04
204675_at	IAA	9.94e-04
219279_at	IAA	9.95e-04
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205292_s_at	IAA	1.028944e-03
202461_at	IAA	1.056849e-03
208686_s_at	IAA	1.056894e-03
211987_at	IAA	1.070779e-03
208791_at	IAA	1.073209e-03
215606_s_at	IAA	1.075892e-03
217815_at	IAA	1.09023e-03
213440_at	IAA	1.108962e-03
216997_x_at	IAA	1.110697e-03
212763_at	IAA	1.17205e-03
210428_s_at	IAA	1.172917e-03
218501_at	IAA	1.182111e-03
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200610_s_at	IAA	1.220998e-03
219470_x_at	IAA	1.256633e-03
208777_s_at	IAA	1.284705e-03
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203537_at	IAA	1.335028e-03
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 218711 _s_at IAH 0.02
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 209716 _at IAH 0.02
 213892 _s_at IAH 0.02
 21822 6 _s_at IAH 0.02
 201685 _s_at IAH 0.02

203853_s_at - i Ah tno 2

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219966_x_at	IAH	0.02	218208__at	IAH	0.03
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203276_at	IAH	0.03	212665_at	IAH	0.03
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200593_s_at	IAH	0.03	207104_x_at	IAH	0.03
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205305_at	IAH	0.03	20262_6_s_at	IAH	0.03
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31845	_at	IAH	0.03	209384_at	IAH	0.03
41160	_at	IAH	0.03	205132_at	IAH	0.03
204436	_at	IAH	0.03	218866_s_at	IAH	0.03
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211561	_x_at	IAH	0.03	218577_at	IAH	0.03
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211924	_s_at	IAH	0.03	35265_at	IAH	0.04
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206036_s_at	IT	0.04							
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220023_at	IT	0.04							
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219933_at	IU	6.32e-04	217764_s_at	IU	9.44e-04
214305_s_at	IU	6.32e-04	204054_at	IU	9.44e-04
218467_at	IU	6.32e-04	203409_at	IU	9.44e-04
218019_s_at	IU	6.32e-04	203922_s_at	IU	9.44e-04
218139_s_at	IU	6.32e-04	211576_s_at	IU	9.44e-04
203621_at	IU	6.32e-04	209555_s_at	IU	9.44e-04
203575_at	IU	6.32e-04	212192_at	IU	9.44e-04
204489_s_at	IU	6.32e-04	202917_s_at	IU	9.44e-04
221597_s_at	IU	6.32e-04	203126_at	IU	9.44e-04
209964_s_at	IU	6.32e-04	203041_s_at	IU	9.44e-04
203067_at	IU	6.32e-04	202856_s_at	IU	9.44e-04
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201413_at	IU	6.32e-04	211684_s_at	IU	9.6e-04
201512_s_at	IU	6.43e-04	200028_s_at	IU	9.69e-04
207100_s_at	IU	6.47e-04	212250_at	IU	9.82e-04
200919_at	IU	6.62e-04	203799_at	IU	9.87e-04
216306_x_at	IU	6.8e-04	206613_s_at	IU	1.02191e-03
204834_at	IU	6.84e-04	201348_at	IU	1.024879e-03
213528_at	IU	6.87e-04	201626_at	IU	1.029633e-03
215096_s_at	IU	6.94e-04	207705_s_at	IU	1.050697e-03
211730_s_at	IU	6.98e-04	220750_s_at	IU	1.066501e-03
213082_s_at	IU	7.06e-04	202369_s_at	IU	1.088391e-03
200812_at	IU	7.08e-04	200768_s_at	IU	1.089403e-03
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201066_at	IU	7.26e-04	203343_at	IU	1.103889e-03
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202189_x_at	IU	8.03e-04	213128_s_at	IU	1.155468e-03
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200703_at	IU	8.6e-04	206050_s_at	IU	1.155468e-03
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200676_s_at	IU	8.6e-04	208905_at	IU	1.155468e-03
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217835_x_at	IU	8.96e-04	201695_s_at	IU	1.155468e-03

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201193_at	IU	3.081956e-03	221495_s_at	IU	3.368092e-03
209186_at	IU	3.09683e-03	212085_at	IU	3.368092e-03
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224599_at	IU	3.113216e-03	201245_s_at	IU	3.368092e-03
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203329_at	IU	3.128505e-03	210371_s_at	IU	3.373734e-03
217974_at	IU	3.139364e-03	202475_at	IU	3.374878e-03
201322_at	IU	3.146199e-03	201797_s_at	IU	3.382258e-03
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200991_s_at	IU	3.170553e-03	206278_at	IU	3.414889e-03
219006_at	IU	3.173393e-03	213735_s_at	IU	3.420671e-03
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217973_at	IU	3.201813e-03	212561_at	IU	3.470973e-03
227442_at	IU	3.20603e-03	220948_s_at	IU	3.505089e-03
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218385_at	IU	3.243383e-03	214259_s_at	IU	3.571851e-03
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 203279_at IU 8.43432e-03
 202698_x_at IU 8.43432e-03
 202420_s_at IU 8.43432e-03
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 210784_x_at IU 8.705515e-03
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 200706_s_at IU 8.779138e-03
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 212144_at IU 8.822637e-03
 208961_s_at IU 8.836469e-03
 217724_at IU 8.839037e-03
 214784_x_at IU 8.883028e-03
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 217852_s_at IU 8.899758e-03
 207668_x_at IU 8.904795e-03
 218319_at IU 8.906323e-03
 220319_s_at IU 8.908593e-03
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221058_s_at	IU	9.035803e-03	202535_at	IU	9.778144e-03
220720_x_at	IU	9.035803e-03	207571_x_at	IU	9.819909e-03
210980_s_at	IU	9.035803e-03	205677_s_at	IU	9.843891e-03
211581_x_at	IU	9.035803e-03	213049_at	IU	9.857778e-03
202775_s_at	IU	9.035803e-03	201521_s_at	IU	9.860417e-03
202812_at	IU	9.035803e-03	243508_at	IU	9.886802e-03
205140_at	IU	9.035803e-03	217104_at	IU	9.897129e-03
207275_s_at	IU	9.035803e-03	217900_at	IU	9.900576e-03
205859_at	IU	9.035803e-03	219129_s_at	IU	9.900655e-03
204971_at	IU	9.035803e-03	203789_s_at	IU	9.907088e-03
200999_s_at	IU	9.035803e-03	207815_at	IU	9.907088e-03
201560_at	IU	9.035803e-03	202001_s_at	IU	9.943522e-03
211594_s_at	IU	9.059466e-03	218281_at	IU	9.966417e-03
201699_at	IU	9.075184e-03	217797_at	IU	9.972582e-03
222985_at	IU	9.083248e-03	201241_at	IU	9.993582e-03
52169_at	IU	9.125119e-03	201192_s_at	IU	9.993785e-03
201113_at	IU	9.125187e-03	207760_s_at	IU	0.01
228452_at	IU	9.151743e-03	209449_at	IU	0.01
212296_at	IU	9.168955e-03	209240_at	IU	0.01
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216232_s_at	IU	9.219771e-03	219512_at	IU	0.01
210811_s_at	IU	9.225131e-03	200043_at	IU	0.01
206707_x_at	IU	9.233802e-03	204218_at	IU	0.01
216979_at	IU	9.243684e-03	220647_s_at	IU	0.01
200959_at	IU	9.250853e-03	218996_at	IU	0.01
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203175_at	IU	9.29554e-03	219013_at	IU	0.01
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205047_s_at	IU	9.360026e-03	210176_at	IU	0.01
203790_s_at	IU	9.364268e-03	221541_at	IU	0.01
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218753_at	IU	9.394571e-03	37965_at	IU	0.01
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203647_s_at	IU	9.42394e-03	204960_at	IU	0.01
202946_s_at	IU	9.452504e-03	218503_at	IU	0.01
217969_at	IU	9.506022e-03	209267_s_at	IU	0.01
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221712_s_at	IU	9.52349e-03	205231_s_at	IU	0.01
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226239_at	IU	9.575008e-03	37201_at	IU	0.01
219244_s_at	IU	9.592608e-03	213936_x_at	IU	0.01
204918_s_at	IU	9.597473e-03	217796_s_at	IU	0.01
204208_at	IU	9.600325e-03	218301_at	IU	0.01
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210283_x_at	IU	9.642105e-03	220605_s_at	IU	0.01
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221568_s_at	IU	9.757457e-03	206689_x_at	IU	0.01
219329_s_at	IU	9.758958e-03	205687_at	IU	0.01

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201063	_at	IU	0.01
202058	_s_at	IU	0.01
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218627	_at	IU	0.01
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Id	Feature	Ref	Seq	Score	Value	Notes
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215399	s_at	IU	0.01			
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222065	s_at	IU	0.01			
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203097	s_at	IU	0.01			
202352	s_at	IU	0.01			
202380	s_at	IU	0.01			
207358	x_at	IU	0.01			
205584	_at	IU	0.01			
205789	_at	IU	0.01			
205128	x_at	IU	0.01			
205025	_at	IU	0.01			
205685	_at	IU	0.01			
205928	_at	IU	0.01			
209433	s_at	IU	0.01			
207707	s_at	IU	0.01			
200714	x_at	IU	0.01			
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201422	_at	IU	0.01			
201921	_at	IU	0.01			
204951	_at	IU	0.01			
224560	_at	IU	0.01			
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200715	x_at	IU	0.01			
227625	s_at	IU	0.01			
238530	_at	IU	0.01			
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209668	x_at	IU	0.01			
203489	_at	IU	0.01			
201683	x_at	IU	0.01			
214459	x_at	IU	0.01			
AFFX-HUMGAPDH/						
M33197		IU	0.01			
208858	s_at	IU	0.01			
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210648	x_at	IU	0.01			
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213209	_at	IU	0.01			
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210453	_x_at	IU	0.01	201361	_at	IU	0.01
202343	_x_at	IU	0.01	201054	_at	IU	0.01
202635	_s_at	IU	0.01	209122	_at	IU	0.01
202221	_s_at	IU	0.01	219889	_at	IU	0.01
202264	_s_at	IU	0.01	218870	_at	IU	0.01
204929	_s_at	IU	0.01	212625	_at	IU	0.01
205967	_at	IU	0.01	212657	_s_at	IU	0.01
209198	_s_at	IU	0.01	214060	_at	IU	0.01
209075	_s_at	IU	0.01	215773	_x_at	IU	0.01
208778	_s_at	IU	0.01	215633	_x_at	IU	0.01
200846	_s_at	IU	0.01	215813	_s_at	IU	0.01
200597	_at	IU	0.01	204774	_at	IU	0.01
201705	_at	IU	0.01	204646	_at	IU	0.01
201272	_at	IU	0.01	203996	_s_at	IU	0.01
200885	_at	IU	0.01	203656	_at	IU	0.01
201722	_s_at	IU	0.01	204204	_at	IU	0.01
218056	_at	IU	0.01	203574	_at	IU	0.01
216241	_s_at	IU	0.01	211101	_x_at	IU	0.01
207078	_at	IU	0.01	202727	_s_at	IU	0.01
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203401	_at	IU	0.01	202442	_at	IU	0.01
202082	_s_at	IU	0.01	202241	_at	IU	0.01
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210582	_s_at	IU	0.01	205922	_at	IU	0.01
206129	_s_at	IU	0.01	208488	_s_at	IU	0.01
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231917	_at	IU	0.01	207730	_x_at	IU	0.01
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208628	_s_at	IU	0.01	200911	_s_at	IU	0.01
223026	_s_at	IU	0.01	219777	_at	IU	0.01
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204286	_s_at	IU	0.01	209247	_s_at	IU	0.01
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				225299	_at	IU	0.01

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208152_s_at	IU	0.04	200744_s_at	IW	1.692302e-03
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206120_at	IW	1.335403e-03	211864_s_at	IW	3.48286e-03
223143_s_at	IW	1.360747e-03	211230_s_at	IW	3.48286e-03
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229933_at	IW	1.508178e-03	211559_s_at	IW	3.48286e-03
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 233292 s at IW 9.909526e-03
 1557158 s at IW 9.935715e-03
 202068 s at IW 9.970776e-03
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 202613 at IW 0.01
 215159 s at IW 0.01
 211574 s at IW 0.01
 1552660 a at IW 0.01
 235222 x at IW 0.01
 202089 s at IW 0.01
 217965 s at IW 0.01
 225800 at IW 0.01
 237338 at IW 0.01
 37966 at IW 0.01
 207223 s at IW 0.01
 228490 at IW 0.01
 1559052 s at IW 0.01
 201536 at IW 0.01
 222099 s at IW 0.01
 203796 s at IW 0.01
 223966 at IW 0.01
 220320 at IW 0.01
 224892 at IW 0.01
 205411 at IW 0.01
 228099 at IW 0.01

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 1552612 : at IW 0.01
 203773 x at IW 0.01
 206184 at IW 0.01
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 202527_s_at IW 0.01
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 220349_s_at IW 0.01
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204132	_s_at	iw	0.02	202650_s_at	IW	0.02
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209674	_at	IW	0.02	1552542_s_at	IW	0.02
226163	_at	IW	0.02	1553723_at	IW	0.02
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201560	_at	IW	0.02	226947_at	IW	0.02
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224221	_s_at	IW	0.02	203145_at	IW	0.02
240288	_at	IW	0.02	202380_s_at	IW	0.02
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212407	_at	IW	0.02	218746_at	IW	0.03
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205750	_at	IW	0.02	208864_s_at	IW	0.03
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224281	_s_at	IW	0.02	1555106_a_at	IW	0.03
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2-i r 7 1 S-at ~5E U.O.T. 2-1

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 208498_s_at 3C 4.86e-06
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217868 _s_at 3C 2.09e-05
230529 _at 3C 2.1e-05
219040 _at 3C 2.13e-05
205370 _x_at 3C 2.2e-05
220046 _s_at 3C 2.3e-05
213347 _x_at 3C 2.3e-05
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243981 _at 3C 2.56e-05
218920 _at 3C 2.65e-05
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236621 _at 3C 2.74e-05
219957 _at 3C 2.74e-05
203900 _at 3C 2.76e-05
212753 _at 3C 2.82e-05
200899 _s_at 3C 2.82e-05
236436 _at 3C 2.94e-05
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203442 _x_at 3C 3.39e-05
201748 _s_at 3C 3.41e-05
209148 _at 3C 3.49e-05
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234671 _at 3C 3.73e-05
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207941 _s_at 3C 4.14e-05
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239740 _at 3C 4.89e-05
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222981 _s_at 3C 5.83e-05
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206035 _at 3C 5.87e-05
AFFX-
hum_alu_at 3C 5.96e-05
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213065 _at 3C 6.06e-05
209409 _at 3C 6.16e-05
212531 _at 3C 6.24e-05
78047 _s_at 3C 6.27e-05
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225995 _x_at 3C 6.57e-05
44146 _at 3C 6.58e-05
214459 _x_at 3C 6.6e-05
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219980 _at 3C 6.72e-05
213624 _at 3C 6.78e-05
202038 _at 3C 6.78e-05
207384 _at 3C 6.86e-05
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219672 _at 3C 7.0e-05
1558111 _at 3C 7.04e-05
213671 _s_at 3C 7.06e-05
220661 _s_at 3C 7.1e-05
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208720 _s_at 3C 7.1e-05
202532 _s_at 3C 7.16e-05
200726 _at 3C 7.16e-05
200998 _s_at 3C 7.18e-05
214414 _x_at 3C 7.2e-05
211749 _s_at 3C 7.29e-05
202917 _s_at 3C 7.32e-05
217928 _s_at 3C 7.35e-05
208039 _at 3C 7.35e-05
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224616 _at 3C 7.68e-05
220446 _s_at 3C 7.82e-05
213867 _x_at 3C 7.91e-05
225756 _at 3C 8.15e-05
201315 _x_at 3C 8.19e-05
205513 _at 3C 8.23e-05
207730 _x_at 3C 8.38e-05
226076 _s_at 3C 8.41e-05
219259 _at 3C 8.43e-05
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208943 _s_at 3C 8.55e-05
213622 _at 3C 8.62e-05
202951 _at 3C 8.8e-05
212661 _x_at 3C 8.88e-05
201475 _x_at 3C 8.88e-05
235479 _at 3C 8.96e-05

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2088'351₃ 3C 9.9e-05
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 202057 _at 3C 9.05e-05
 200801 _x_at 3C 9.07e-05
 235816 _s_at 3C 9.14e-05
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 210142 _x_at 3C 9.59e-05
 201136 _at 3C 9.62e-05
 202880 _s_at 3C 9.73e-05
 221858 _at 3C 9.74e-05
 212024 _x_at 3C 9.78e-05
 201554 _x_at 3C 9.79e-05
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 220034 _at 3C 1.03e-04
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 203107 _x_at 3C 1.06e-04
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 203922_s_at 3C 4.11e-04
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205237_at 3C 4.85e-04
227207_x_at 3C 4.85e-04
212271_at 3C 4.9e-04
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220945_x_at 3C 4.92e-04
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223993_s_at 3C 5.02e-04
226566_at 3C 5.02e-04
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200999_s_at 3C 5.08e-04
89476_r_at 3C 5.12e-04
91952_at 3C 5.15e-04
213340_s_at 3C 5.15e-04
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224414_s_at 3C 5.27e-04
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209771_x_at 3C 5.32e-04
235396_at 3C 5.33e-04
201914_s_at 3C 5.35e-04
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71933_at 3C 5.36e-04
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21197_8_x_at 3C 5.46e-04
219095_at 3C 5.5e-04
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225289_at 3C 5.53e-04
225830_at 3C 5.54e-04
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1554510_s_at 3C 5.98e-04
204308_s_at 3C

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204520_x_at 3C

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203560	_at	3D	2.43e-07
201057	_s_at	3D	2.48e-07
203744	_at	3D	4.13e-07
219421	_at	3D	4.41e-07
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203723	_at	3D	7.04e-07
238701	_x_at	3D	7.87e-07
91682	_at	3D	7.9e-07
213545	_x_at	3D	1.47e-06
202104	_s_at	3D	1.71e-06
1553718	_at	3D	1.73e-06
227833	_s_at	3D	1.75e-06
208246	_x_at	3D	1.91e-06
1562511	_at	3D	1.91e-06
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225787	_at	3D	2.09e-06
244756	_at	3D	2.34e-06
204404	_at	3D	2.59e-06
213932	_x_at	3D	3.03e-06
234631	_at	3D	3.58e-06
206044	_s_at	3D	3.66e-06
208781	_x_at	3D	3.83e-06
204158	_s_at	3D	4.11e-06
220071	_x_at	3D	4.92e-06
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202180	_s_at	3D	5.2e-06
1563497	_at	3D	6.13e-06
200871	_s_at	3D	6.22e-06
214003	_x_at	3D	6.47e-06
234981	_x_at	3D	6.64e-06
201599	_at	3D	6.8e-06
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222311	_s_at	3D	7.78e-06
219981	_x_at	3D	8.34e-06
213836	_s_at	3D	8.49e-06
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1552302	_at	3D	8.68e-06

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201010	_s_at	3D	1.06e-05
218157	_x_at	3D	1.12e-05
224564	_s_at	3D	1.13e-05
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202157	_s_at	3D	1.14e-05
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220000	_at	3D	1.24e-05
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236436	_at	3D	2.94e-05
219594	_at	3D	3.05e-05
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209430	_at	3D	3.22e-05
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203442	_x_at	3D	3.39e-05
201748	_s_at	3D	3.41e-05
206177	_s_at	3D	3.55e-05
201972	_at	3D	3.6e-05
206676	_at	3D	3.61e-05
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210981	_s_at	3D	3.65e-05
204018	_x_at	3D	3.7e-05
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205312	_at	3D	3.95e-05
207474	_at	3D	3.95e-05
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202460	_s_at	3D	4.17e-05

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 201055 _s_at 3D 4.54e-05
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 200021 _at 3D 4.6e-05
 203925 _at 3D 4.77e-05
 226091 _s_at 3D 4.89e-05
 239740 _at 3D 4.89e-05
 202957 _at 3D 4.93e-05
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 205583 _s_at 3D 5.28e-05
 231735 _s_at 3D 5.33e-05
 205716 _at 3D 5.67e-05
 225191 _at 3D 5.68e-05
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 204425 _at 3D 5.83e-05
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 207730 _x_at 3D 8.38e-05

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 202057 _at 3D 9.05e-05
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 203107 _x_at 3D 1.06e-04
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 225563 _at 3D 1.09e-04
 222605 _at 3D 1.09e-04
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 M33197 3D 1.6e-04
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 74694_s_at 3D 1.8e-04
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 204526_s_at 3D 6.43e-04
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203282_at	3E	1.86e-04
1553569_at	3E	1.87e-04
201492_s_at	3E	1.87e-04
202466_at	3E	1.88e-04
206257_at	3E	1.91e-04
208651_x_at	3E	1.94e-04
224250_s_at	3E	1.99e-04
37652_at	3E	2.0e-04
213280_at	3E	2.01e-04

208610_s_at	3E	2.01e-04
1552553_a_at	3E	2.01e-04
218600_at	3E	2.03e-04
201576_s_at	3E	2.08e-04
203535_at	3E	2.09e-04
227094_at	3E	2.1e-04
218039_at	3E	2.11e-04
201071_x_at	3E	2.11e-04
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218599_at	3E	2.13e-04
215832_x_at	3E	2.15e-04
211883_x_at	3E	2.16e-04
200059_s_at	3E	2.16e-04
206790_s_at	3E	2.17e-04
201903_at	3E	2.17e-04
202607_at	3E	2.21e-04
203752_s_at	3E	2.22e-04
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202388_at	3E	2.22e-04
208695_s_at	3E	2.22e-04
232008_s_at	3E	2.24e-04
200663_at	3E	2.26e-04
211275_s_at	3E	2.32e-04
205557_at	3E	2.33e-04
214523_at	3E	2.37e-04
202548_s_at	3E	2.38e-04
202379_s_at	3E	2.38e-04
205068_s_at	3E	2.4e-04
201721_s_at	3E	2.41e-04
221804_s_at	3E	2.43e-04
210069_at	3E	2.43e-04
201605_x_at	3E	2.44e-04
209806_at	3E	2.45e-04
202187_s_at	3E	2.45e-04
202682_s_at	3E	2.45e-04
219033_at	3E	2.46e-04
203318_s_at	3E	2.46e-04
212307_s_at	3E	2.49e-04
205554_s_at	3E	2.49e-04
225640_at	3E	2.5e-04
209762_x_at	3E	2.5e-04
225247_at	3E	2.51e-04
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221580_s_at	3E	2.54e-04
1554892_a_at	3E	2.56e-04
213515_x_at	3E	2.6e-04
218578_at	3E	2.63e-04
203110_at	3E	2.67e-04
203254_s_at	3E	2.7e-04
212222_at	3E	2.73e-04
236165_at	3E	2.8e-04
36030_at	3E	2.81e-04
202990_at	3E	2.83e-04
213198_at	3E	2.91e-04
38710_at	3E	2.92e-04
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220467_at	3E	2.96e-04
206240_s_at	3E	2.98e-04
1554503_a_at	3E	2.98e-04
220416_at	3E	2.99e-04
37028_at	3E	3.0e-04

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214084	_x_at	3E	3.16e-04
218231	_at	3E	3.16e-04
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1557915	_s_at	3E	3.18e-04
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213119	_at	3E	3.21e-04
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213018	_at	3E	3.25e-04
225294	_s_at	3E	3.3e-04
218950	_at	3E	3.3e-04
223097	_at	3E	3.35e-04
208012	_x_at	3E	3.35e-04
202681	_at	3E	3.37e-04
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220408	_x_at	3E	3.42e-04
204192	_at	3E	3.42e-04
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201356	_at	3E	3.45e-04
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211040	_x_at	3E	3.49e-04
211779	_x_at	3E	3.49e-04
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226177	_at	3E	3.58e-04
207674	_at	3E	3.58e-04
213341	_at	3E	3.64e-04
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222667	_s_at	3E	3.68e-04
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200791	_s_at	3E	3.72e-04
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220615	_s_at	3E	3.84e-04
201357	_s_at	3E	3.88e-04
201716	_at	3E	3.89e-04
217732	_s_at	3E	3.9e-04
221547	_at	3E	3.91e-04
221875	_x_at	3E	3.93e-04
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207243	_s_at	3E	3.97e-04
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40149	_at	3E	4.01e-04
229723	_at	3E	4.08e-04
57539	_at	3E	4.08e-04

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211983	_x_at	3E	4.15e-04
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201118	_at	3E	4.24e-04
211940	_x_at	3E	4.3e-04
228336	_at	3E	4.32e-04
225132	_at	3E	4.37e-04
221607	_x_at	3E	4.38e-04
235568	_at	3E	4.41e-04
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222825	_at	3E	4.64e-04
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225251	_at	3E	4.7e-04
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211581	_x_at	3E	4.84e-04
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233072	_at	3E	4.94e-04
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222447	_at	3E	4.94e-04
212015	_x_at	3E	4.98e-04
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89476	_r_at	3E	5.08e-04
91952	_at	3E	5.12e-04
213340	_s_at	3E	5.15e-04
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222262	_s_at	3E	5.17e-04
201096	_s_at	3E	5.19e-04
201550	_x_at	3E	5.19e-04
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200634	_at	3E	5.23e-04
224414	_s_at	3E	5.27e-04
218543	_s_at	3E	5.27e-04
209771	_x_at	3E	5.28e-04
235396	_at	3E	5.32e-04
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227379	_at	3E	5.35e-04
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211978	_x_at	3E	5.37e-04
219095	_at	3E	5.46e-04
225076	_s_at	3E	5.5e-04

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200863_s_at	3E	5.54e-04
205142_x_at	3E	5.58e-04
219281_at	3E	5.68e-04
244495_x_at	3E	5.73e-04
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213483_at	3E	5.75e-04
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208625_s_at	3E	5.82e-04
204308_s_at	3E	5.98e-04
200890_s_at	3E	5.98e-04
218303_x_at	3E	6.04e-04
201768_s_at	3E	6.04e-04
204031_s_at	3E	6.12e-04
1553567_s_at	3E	6.14e-04
201531_at	3E	6.19e-04
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1554624_a_at	3E	6.21e-04
208632_at	3E	6.25e-04
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226544_x_at	3E	6.32e-04
227236_at	3E	6.32e-04
206302_s_at	3E	6.32e-04
208904_s_at	3E	6.32e-04
204526_s_at	3E	6.43e-04
218456_at	3E	6.44e-04
52169_at	3E	6.48e-04
212259_s_at	3E	6.48e-04
202119_s_at	3E	6.54e-04
204211_x_at	3E	6.55e-04
208642_s_at	3E	6.58e-04
212330_at	3E	6.62e-04
213084_x_at	3E	6.68e-04
203012_x_at	3E	6.68e-04
209286_at	3E	6.72e-04
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219403_s_at	3E	6.76e-04
266_s_at	3E	6.77e-04
215089_s_at	3E	6.77e-04
217918_at	3E	6.81e-04
218290_at	3E	6.85e-04
203514_at	3E	6.85e-04
218639_s_at	3E	6.87e-04
222133_s_at	3E	6.87e-04
201232_s_at	3E	6.87e-04
221698_s_at	3E	6.93e-04
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224873_s_at	3E	6.96e-04
225282_at	3E	6.97e-04
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201094_at	3E	6.97e-04
221484_at	3E	7.08e-04
209515_s_at	3E	7.08e-04
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203116_s_at	3E	7.1e-04
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235067_at	3E	7.11e-04
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226050_at	3E	7.13e-04

212460_at	3E	7.13e-04
209369_at	3E	7.13e-04
215313_x_at	3E	7.17e-04
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206656_s_at	3E	7.34e-04
233929_x_at	3E	7.36e-04
201082_s_at	3E	7.37e-04
214550_s_at	3E	7.47e-04
212329_at	3E	7.54e-04
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200958_s_at	3E	7.68e-04
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1554679_a_at	3E	7.71e-04
206738_at	3E	7.75e-04
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203175_at	3E	8.01e-04
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209879_at	3E	8.2e-04
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208643_s_at	3E	8.2e-04
209791_at	3E	8.22e-04
219191_s_at	3E	8.24e-04
203568_s_at	3E	8.26e-04
57739_at	3E	8.29e-04
222557_at	3E	8.44e-04
228230_at	3E	8.54e-04
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210070_s_at	3E	8.79e-04
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218431_at	3E	8.85e-04
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203186_s_at	3E	8.88e-04
202381_at	3E	8.91e-04
201665_x_at	3E	8.95e-04
203750_s_at	3E	8.99e-04
218728_s_at	3E	9.0e-04
207667_s_at	3E	9.0e-04
225899_x_at	3E	9.01e-04
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216379_x_at	3E	9.15e-04
206707_x_at	3E	9.15e-04
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220001_at	3E	9.2e-04
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205277_at	3E	9.35e-04
213261_at	3E	9.38e-04

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228993	_s_at	3E	9.47e-04	202161	_at	3E	1.095152e-03
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210716	_s_at	3E	9.64e-04	244317	_at	3E	1.136175e-03
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214775	_at	3E	9.72e-04	217783	_s_at	3E	1.159664e-03
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223044	_at	3E	9.89e-04	227286	_at	3E	1.188808e-03
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1554899	_s_at	3E	1.00444e-03	208834	_x_at	3E	1.22053e-03
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224680	_at	3E	1.009498e-03	223980	_s_at	3E	1.226563e-03
221480	_at	3E	1.009891e-03	226629	_at	3E	1.226563e-03
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227658	_s_at	3E	1.030586e-03	215706	_x_at	3E	1.23129e-03
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200808	_s_at	3E	1.042669e-03	203281	_s_at	3E	1.243004e-03
220525	_s_at	3E	1.047745e-03	212501	_at	3E	1.245744e-03
214606	_at	3E	1.055733e-03	226739	_at	3E	1.250968e-03
48659	_at	3E	1.057067e-03	202510	_s_at	3E	1.250968e-03
212252	_at	3E	1.058923e-03	214721	_x_at	3E	1.255373e-03
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211919	_s_at	3E	1.069845e-03	200625	_s_at	3E	1.255568e-03
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226726	_at	3E	1.074738e-03	213702	_x_at	3E	1.262895e-03
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236267 _at 31 0.04
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214551 _s_at 31 0.04
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220711 _at 31 0.04
209025 _s_at 31 0.04
223212 _at 31 0.04
1554411 _at 31 0.04
208447 _s_at 31 0.04
214496 _x_at 31 0.04
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205349 _at 3K 1.37e-04
205770 _at 3K 1.82e-04
209099 _x_at 3K 2.99e-04
206039 _at 3K 3.47e-04
216945 _x_at 3K 5.04e-04
201626 _at 3K 1.029633e-03
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201693 _s_at 3K 1.116156e-03
209360 _s_at 3K 1.303325e-03
201625 _s_at 3K 1.47524e-03
203504 _s_at 3K 1.720204e-03
220742 _s_at 3K 1.768548e-03

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" 2 4 15 6 "at "3R "2 :Ü S79"22'e-03
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208763 _s_at 3K 3.978598e-03
204254 _s_at 3K 4.393802e-03
201328 _at 3K 4.84355e-03
204748 _at 3K 6.008283e-03
207075 _at 3K 6.143416e-03
218515 _at 3K 6.148687e-03
210264 _at 3K 7.00613e-03
221867 _at 3K 7.080955e-03
206834 _at 3K 7.65295e-03
219426 _at 3K 7.683749e-03
214762 _at 3K 7.837633e-03
218310 _at 3K 8.034698e-03
200953 _s_at 3K 8.493784e-03
208961 _s_at 3K 8.836469e-03
206488 _s_at 3K 8.955465e-03
216979 _at 3K 9.243684e-03
210742 _at 3K 0.01
219423 _x_at 3K 0.01
221060 _s_at 3K 0.01
209385 _s_at 3K 0.01
202435 _s_at 3K 0.01
203297 _s_at 3K 0.01
210017 _at 3K 0.01
220306 _at 3K 0.01
204805 _s_at 3K 0.01
220800 _s_at 3K 0.01
205896 _at 3K 0.01
204440 _at 3K 0.01
212914 _at 3K 0.01
210847 _x_at 3K 0.01
208026 _at 3K 0.01
222021 _x_at 3K 0.01
218033 _s_at 3K 0.01
214508 _x_at 3K 0.01
210458 _s_at 3K 0.02
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214643 _x_at 3K 0.02
208448 _x_at 3K 0.02
213153 _at 3K 0.02
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47571 _at 3K 0.02
204735 _at 3K 0.02
212737 _at 3K 0.02
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205255 _x_at 3K 0.03
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218476 _at 3K 0.03
202875 _s_at 3K 0.03
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203434 _s_at 3K 0.04
203060 _s_at 3K 0.04
209421 _at 3K 0.04

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219248 _at 3K 0.04
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 210742 _at 3P 3.46e-04
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 222745 _s_at 3P 8.8e-04
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 201631 _s_at 3P 9.461623e-03
 225945 _at 3P 9.682531e-03
 224513 _s_at 3P 9.739683e-03

! " # \$ % & ' () * + , - . / : ; < = > ? @ A B C D E F G H I J K L M N O P Q R S T U V W X Y Z [\] ^ _ ` a b c d e f g h i j k l m n o p q r s t u v w x y z { | } ~ - 9 r g 3 2 1 ' 7 4 e " - 0 3

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 242916 _at 3P 0.02
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 209083_at 3R 0.01
 217232_x_at 3R 0.01
 201376_s_at 3R 0.01
 213453_x_at 3R 0.01
 213039_at 3R 0.01
 212330_at 3R 0.01
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 37950_at 3R 0.01
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 200802_at 3R 0.01
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 202917_s_at 3R 0.01
 202659_at 3R 0.01
 202173_s_at 3R 0.01
 219165_at 3R 0.01
 203258_at 3R 0.01
 225614_at 3R 0.01
 213738_s_at 3R 0.01
 201967_at 3R 0.01
 221744_at 3R 0.01
 201475_x_at 3R 0.01

2 tsirjat_{hum} 3fr 0.02

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204861_s_at 3R 0.02

214582_at 3R 0.02

235388_at 3R 0.02

205074_at 3R 0.02

217356_s_at 3R 0.02

218724_s_at 3R 0.02

201231_s_at 3R 0.02

220796_x_at 3R 0.02

225819_at 3R 0.02

71933_at 3R 0.02

1554770_x_at 3R 0.02

226283_at 3R 0.02

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224693_at 3R 0.02

20121_β_at 3R 0.02

235327_x_at 3R 0.02

219206_x_at 3R 0.02

220661_s_at 3R 0.02

236027_at 3R 0.02

31845_at 3R 0.02

201784_s_at 3R 0.02

209134_s_at 3R 0.02

209069_s_at 3R 0.02

218803_at 3R 0.02

217978_s_at 3R 0.02

200674_s_at 3R 0.02

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208692_at 3R 0.02

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211015_s_at 3R 0.02

221565_s_at 3R 0.02

227388_at 3R 0.02

212312_at 3R 0.02

207289_at 3R 0.02

223303_at 3R 0.02

204351_at 3R 0.02

219690_at 3R 0.02

210504_at 3R 0.02

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218649_x_at 3R 0.02

208961_s_at 3R 0.02

202757_at 3R 0.02

209188_x_at 3R 0.02

AFFX-

hum_alu_at 3R 0.02

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213671_s_at 3R 0.02

208997_s_at 3R 0.02

202779_s_at 3R 0.02

208812_x_at 3R 0.02

202915_s_at 3R 0.02

218298_s_at 3R 0.02

224859_at 3R 0.02

201743_at 3R 0.02

229563_s_at 3R 0.02

218454_at 3R 0.02

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214054_at 3R 0.03

242139_s_at 3R 0.03

201214_s_at 3R 0.03

211004_s_at 3R 0.03

211521_s_at 3R 0.03

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223283_s_at 3R 0.03

204848_x_at 3R 0.03

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225649_s_at 3R 0.03

213957_s_at 3R 0.03

200853_at 3R 0.03

204336_s_at 3R 0.03

201950_x_at 3R 0.03

AFFX-HUMGAPDH/

M33197 3R 0.03

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218111_s_at 3R 0.03

214049_x_at 3R 0.03

223637_s_at 3R 0.03

226994_at 3R 0.03

222623_s_at 3R 0.03

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200866_s_at 3R 0.03

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205842_s_at 3R 0.03

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218249_at 3R 0.03

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218198_at 3R 0.04

212869_x_at 3R 0.04

200964_at 3R 0.04

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206222_at 3R 0.04

208018_s_at 3R 0.04

203900_at 3R 0.04

209694_at 3R 0.04

211316_x_at 3R 0.04

207674_at 3R 0.04

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209265_s_at 3R 0.04

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208610_s_at 3R 0.04

202484_s_at 3R 0.04

1558136_s_at 3R 0.04

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203309_s_at 3R 0.04

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"20*278 9-*St:**** **4D' -CiroE" ---

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" 2-2 ÖÖor.ät " 4T myr Ür " r"

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g. 02749 at 0.01

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 220001 _at 4H 0.01

PCT/US2005/022071

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226119	"_at	4H	0.01
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232843	"_s_at	4H	0.01
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241937	"_s_at	4H	0.01
212500	_at	4H	0.01
218263	_s_at	4H	0.01
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AFFX-KUMGAPDH/			
M33197		4H	0.01
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213453	"_x_at	4H	0.01
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216237	_s_at	4H	0.01
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222537	"_s_at	4H	0.01
222642	"_s_at	4H	0.01
212242	"_at	4H	0.01

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 1552798_a_at 4H 0.01
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 201412_at 4H 0.01
 201463_s_at 4H 0.01
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 155862C)_at 4H 0.01
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 204562_at 4H 0.01
 235298_at 4H 0.01
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 226970_at 4H 0.01
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 201874_at 4H 0.01
 220583_at 4H 0.01
 225403_at 4H 0.01
 1554500_a_at 4H 0.01
 221401_at 4H 0.01

"in 89io" _at_ is 0.01

218526	_s_at	4H	0.01	233979	_s_at	4H	0.01
228393	_s_at	4H	0.01	200674	_s_at	4H	0.01
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223634	_at	4H	0.01	201585	_s_at	4H	0.01
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221257	_x_at	4H	0.01	206643	_at	4H	0.01
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201745	_at	4H	0.01	228318	_s_at	4H	0.01
218007	_s_at	4H	0.01	220005	_at	4H	0.01
201010	_s_at	4H	0.01	212360	_at	4H	0.01
221492	_s_at	4H	0.01	212264	_s_at	4H	0.01
201783	_s_at	4H	0.01	212794	_s_at	4H	0.01
201594	_s_at	4H	0.01	213133	_s_at	4H	0.01
232104	_at	4H	0.01	218289	_s_at	4H	0.01
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218629	_at	4H	0.01	204222	_s_at	4H	0.01
230308	_at	4H	0.01	204638	_at	4H	0.01
32811	_at	4H	0.01	221425	_s_at	4H	0.01
219681	_s_at	4H	0.01	220691	_at	4H	0.01
200798	_x_at	4H	0.01	221511	_x_at	4H	0.01
232114	_at	4H	0.01	223167	_s_at	4H	0.01
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216234	_s_at	4H	0.01	203005	_at	4H	0.01
207 676	_at	4H	0.01	205306	_x_at	4H	0.01
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213378	_s_at	4H	0.01	214321	_at	4H	0.01
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213160	_at	4H	0.01	203023	_at	4H	0.02
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230879	_at	4H	0.01	1553868	_a_at	4H	0.02
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				223531	_x_at	4H	0.02

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 202428_x_at 4H 0.02
 212333_at 4H 0.02

2-253'84 at 4H 0.02
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 218603 at 4H 0.02
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 214705 at 4H 0.02
 222191 s at 4H 0.02
 204370 at 4H 0.02
 232602 at 4H 0.02
 1559138 a at 4H 0.02
 201197 at 4H 0.02
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 213579 s at 4H 0.02
 215984 s at 4H 0.02
 203634 s at 4H 0.02
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 200884 at 4H 0.02
 224406 s at 4H 0.02
 203430 at 4H 0.02
 207636 at 4H 0.02
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 1552625 a at 4H 0.02
 224575 at 4H 0.02
 223313 s at 4H 0.02
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 205654 at 4H 0.02
 1552274 at 4H 0.02
 37966 at 4H 0.02

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 55705 at 4H 0.02
 53912 at 4H 0.02
 219049 at 4H 0.02
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 215165 x at 4H 0.02
 218107 at 4H 0.02

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 227445_at 4H 0.03
 200945_s_at 4H 0.03
 213073_at 4H 0.03
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 1557657_a_at 4H 0.03
 218295_s_at 4H 0.03
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 215093_at 4H 0.03
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 222702_x_at 4H 0.03
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 202955_s_at 4H 0.03
 214084_x_at 4H 0.03
 200065_s_at 4H 0.03
 202345_s_at 4H 0.03
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 228597_at 4H 0.03
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 220316_at 4H 0.03
 230266_at 4H 0.03
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 209772_s_at 4H 0.03
 210931_at 4H 0.03
 211594_s_at 4H 0.03
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 201685_s_at 4H 0.03
 219971_at 4H 0.03
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 209640_at 4H 0.03
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 203887_s_at 4H 0.03
 204573_at 4H 0.03
 220213_at 4H 0.03
 201921_at 4H 0.03
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 225434_at 4H 0.03
 201541_s_at 4H 0.03
 210774_s_at 4H 0.03
 218011_at 4H 0.03
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 204996_s_at 4H 0.03
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 222990_at 4H 0.03
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 212133_at 4H 0.03
 227964_at 4H 0.03
 206498_at 4H 0.03
 235048_at 4H 0.03
 91682_at 4H 0.03
 219922_s_at 4H 0.03
 202832_at 4H 0.03
 1568720_at 4H 0.03
 211004_s_at 4H 0.03
 230707_at 4H 0.03

209606_at 4H 0.03
 1555245_s_at 4H 0.03
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 209383_at 4H 0.03
 228053_s_at 4H 0.03
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 206072_at 4H 0.03
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 220062_s_at 4H 0.03
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 200924_s_at 4H 0.03
 201043_s_at 4H 0.03
 206830_at 4H 0.03
 219326_s_at 4H 0.03
 220138_at 4H 0.03
 218178_s_at 4H 0.03
 211765_x_at 4H 0.03
 238817_at 4H 0.03
 203199_s_at 4H 0.03
 216316_x_at 4H 0.03
 217014_s_at 4H 0.03
 203115_at 4H 0.03
 205731_s_at 4H 0.03
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 213995_at 4H 0.03
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 203814_s_at 4H 0.03
 222071_s_at 4H 0.03
 222422_s_at 4H 0.03
 210582_s_at 4H 0.03
 211795_s_at 4H 0.03
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 203090_at 4H 0.03
 202197_at 4H 0.03
 205060_at 4H 0.03
 206861_s_at 4H 0.03
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 209218_at 4H 0.03
 207809_s_at 4H 0.03
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 1569257_at 4H 0.03
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 201096_s_at 4H 0.03
 201928_at 4H 0.03
 209863_s_at 4H 0.03
 208104_s_at 4H 0.03
 225201_s_at 4H 0.03
 220241_at 4H 0.03
 219810_at 4H 0.03

21*970 (T) 4H (TT) 4H
 207414 _s_at 4H 0.03
 213603 _s_at 4H 0.03
 203926 _x_at 4H 0.03
 210004 _at 4H 0.03
 229983 _at 4H 0.03
 207104 _x_at 4H 0.03
 201863 _at 4H 0.03
 221520 _s_at 4H 0.03
 203583 _at 4H 0.03
 209409 _at 4H 0.03
 209879 _at 4H 0.03
 1554873 _at 4H 0.03
 207419 _s_at 4H 0.03
 225470 _at 4H 0.03
 1558330 _x_at 4H 0.03
 203536 _s_at 4H 0.03
 1552695 _a_at 4H 0.03
 209639 _s_at 4H 0.03
 202807 _s_at 4H 0.03
 222969 _at 4H 0.03
 214844 _s_at 4H 0.03
 1552338 _at 4H 0.03
 222248 _s_at 4H 0.03
 207028 _at 4H 0.03
 220327 _at 4H 0.03
 201658 _at 4H 0.03
 211581 _x_at 4H 0.03
 213823 _at 4H 0.03
 57532 _at 4H 0.03
 208746 _x_at 4H 0.03
 207253 _s_at 4H 0.03
 1569102 _at 4H 0.03
 213545 _x_at 4H 0.03
 200078 _s_at 4H 0.03
 207785 _s_at 4H 0.03
 228916 _at 4H 0.03
 224840 _at 4H 0.03
 236848 _s_at 4H 0.03
 228845 _at 4H 0.03
 202449 _s_at 4H 0.03
 221179 _at 4H 0.03
 234695 _x_at 4H 0.03
 220490 _at 4H 0.03
 213129 _s_at 4H 0.03
 219890 _at 4H 0.03
 221277 _s_at 4H 0.03
 213063 _at 4H 0.03
 213707 _s_at 4H 0.03
 1553627 _s_at 4H 0.03
 206973 _at 4H 0.03
 226881 _at 4H 0.03
 205995 _x_at 4H 0.03
 205263 _at 4H 0.03
 213434 _at 4H 0.03
 203469 _s_at 4H 0.03
 211540 _s_at 4H 0.03
 206638 _at 4H 0.03
 233150 _at 4H 0.03
 223141 _at 4H 0.03
 209270 _at 4H 0.03
 202220 _at 4H 0.03
 225320 _at 4H 0.03
 239377 _at 4H 0.03

224719 _s_at 4H 0.03
 226422 _at 4H 0.03
 208389 _s_at 4H 0.03
 203936 _s_at 4H 0.03
 156403 _a_at 4H 0.03
 200665 _s_at 4H 0.03
 200981 _x_at 4H 0.03
 202021 _x_at 4H 0.03
 219398 _at 4H 0.03
 208138 _at 4H 0.03
 206816 _s_at 4H 0.03
 1555725 _a_at 4H 0.03
 219656 _at 4H 0.03
 203919 _at 4H 0.03
 204624 _at 4H 0.03
 1553296 _at 4H 0.03
 1553438 _at 4H 0.03
 204326 _x_at 4H 0.03
 1555729 _a_at 4H 0.03
 210937 _s_at 4H 0.03
 202360 _at 4H 0.03
 208878 _s_at 4H 0.03
 206662 _at 4H 0.03
 218854 _at 4H 0.03
 223132 _s_at 4H 0.03
 202509 _s_at 4H 0.03
 212100 _s_at 4H 0.03
 208428 _at 4H 0.03
 216418 _at 4H 0.03
 209517 _s_at 4H 0.03
 212933 _x_at 4H 0.03
 234784 _at 4H 0.03
 202921 _s_at 4H 0.03
 1554769 _at 4H 0.03
 232263 _at 4H 0.03
 210975 _x_at 4H 0.03
 201352 _at 4H 0.03
 219923 _at 4H 0.03
 200091 _s_at 4H 0.03
 202968 _s_at 4H 0.03
 231718 _at 4H 0.03
 227266 _s_at 4H 0.03
 208052 _x_at 4H 0.03
 203484 _at 4H 0.03
 213892 _s_at 4H 0.03
 218905 _at 4H 0.03
 243623 _at 4H 0.03
 1563657 _at 4H 0.03
 1569631 _at 4H 0.03
 220460 _at 4H 0.03
 221399 _at 4H 0.03
 231798 _at 4H 0.03
 208903 _at 4H 0.03
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 209249 _s_at 4H 0.03
 212790 _x_at 4H 0.03
 200082 _s_at 4H 0.03
 203535 _at 4H 0.03
 215236 _s_at 4H 0.03
 234093 _at 4H 0.03
 200823 _x_at 4H 0.03
 202026 _at 4H 0.03
 202277 _at 4H 0.03
 219631 _at 4H 0.03

"2'04 959 _s_at " 4 F " 0.03"

206796 _at 4H 0.03
 234988 _at 4H 0.03
 206959 _s_at 4H 0.03
 212332 _at 4H 0.03
 1555349 _a_at 4H 0.03
 1567284 _at 4H 0.03
 1560974 _s_at 4H 0.03
 202729 _s_at 4H 0.03
 205720 _at 4H 0.03
 208742 _s_at 4H 0.03
 220621 _at 4H 0.03
 227240 _at 4H 0.03
 221854 _at 4H 0.03
 203460 _s_at 4H 0.03
 207351 _s_at 4H 0.03
 201740 _at 4H 0.03
 223151 _at 4H 0.03
 214179 _s_at 4H 0.03
 223135 _s_at 4H 0.03
 207338 _s_at 4H 0.03
 200791 _s_at 4H 0.03
 221613 _s_at 4H 0.03
 1553579 _a_at 4H 0.03
 207254 _at 4H 0.03
 214181 _x_at 4H 0.03
 221090 _s_at 4H 0.03
 211250 _s_at 4H 0.03
 210646 _x_at 4H 0.03
 226531 _at 4H 0.03
 226042 _at 4H 0.03
 212836 _at 4H 0.03
 219737 _s_at 4H 0.03
 205567 _at 4H 0.03
 208076 _at 4H 0.03
 200683 _s_at 4H 0.03
 213940 _s_at 4H 0.03
 208948 _s_at 4H 0.03
 204178 _s_at 4H 0.03
 1553863 _at 4H 0.03
 220526 _s_at 4H 0.03
 43544 _at 4H 0.03
 224078 _at 4H 0.03
 211964 _at 4H 0.03
 202483 _s_at 4H 0.03
 220195 _at 4H 0.03
 218010 _x_at 4H 0.03
 208042 _at 4H 0.03
 243456 _at 4H 0.03
 223620 _at 4H 0.04
 1553770 _a_at 4H 0.04
 238803 _at 4H 0.04
 1553087 _at 4H 0.04
 223706 _at 4H 0.04
 38447 _at 4H 0.04
 222678 _s_at 4H 0.04
 201227 _s_at 4H 0.04
 226100 _at 4H 0.04
 221503 _s_at 4H 0.04
 240603 _s_at 4H 0.04
 223011 _s_at 4H 0.04
 225651 _at 4H 0.04
 242961 _x_at 4H 0.04
 219008 _at 4H 0.04

219284 _at 4H 0.04
 212487 _at 4H 0.04
 213261 _at 4H 0.04
 214170 _x_at 4H 0.04
 215051 _x_at 4H 0.04
 218316 _at 4H 0.04
 217943 _s_at 4H 0.04
 203254 _s_at 4H 0.04
 221905 _at 4H 0.04
 220761 _s_at 4H 0.04
 210119 _at 4H 0.04
 211794 _at 4H 0.04
 204980 _at 4H 0.04
 205953 _at 4H 0.04
 206245 _s_at 4H 0.04
 20664 9_s_at 4H 0.04
 209088 _s_at 4H 0.04
 208591 _s_at 4H 0.04
 209221 _s_at 4H 0.04
 209131 _s_at 4H 0.04
 209026 _x_at 4H 0.04
 1552703 _s_at 4H 0.04
 1557227 _s_at 4H 0.04
 200776 _s_at 4H 0.04
 201060 _x_at 4H 0.04
 201805 _at 4H 0.04
 202635 _s_at 4H 0.04
 205051 _s_at 4H 0.04
 207990 _x_at 4H 0.04
 219762 _s_at 4H 0.04
 200713 _s_at 4H 0.04
 203672 _x_at 4H 0.04
 209962 _at 4H 0.04
 208766 _s_at 4H 0.04
 202084 _s_at 4H 0.04
 224395 _s_at 4H 0.04
 209211 _at 4H 0.04
 1569932 _at 4H 0.04
 229770 _at 4H 0.04
 226319 _s_at 4H 0.04
 219933 _at 4H 0.04
 221208 _s_at 4H 0.04
 213492 _at 4H 0.04
 218850 _s_at 4H 0.04
 205569 _at 4H 0.04
 210341 _at 4H 0.04
 208827 _at 4H 0.04
 235202 _x_at 4H 0.04
 222636 _at 4H 0.04
 206917 _at 4H 0.04
 1553137 _s_at 4H 0.04
 200818 _at 4H 0.04
 226036 _x_at 4H 0.04
 208360 _s_at 4H 0.04
 220774 _at 4H 0.04
 227724 _at 4H 0.04
 219329 _s_at 4H 0.04
 228531 _at 4H 0.04
 240159 _at 4H 0.04
 202991 _at 4H 0.04
 204602 _at 4H 0.04
 233408 _at 4H 0.04
 206864 _s_at 4H 0.04
 227063 _at 4H 0.04

22t) 99frvs+ie- n⁴ 4H -d'ToT # --dlw

223398	_at	4H	0.04
35254	_at	4H	0.04
1554085	_at	4H	0.04
208061	_at	4H	0.04
235258	_at	4H	0.04
217865	_at	4H	0.04
210137	_s_at	4H	0.04
220819	_at	4H	0.04
210303	_at	4H	0.04
201605	_x_at	4H	0.04
204864	_s_at	4H	0.04
204538	_x_at	4H	0.04
203490	_at	4H	0.04
1561429	_a_at	4H	0.04
202422	_s_at	4H	0.04
215299	_x_at	4H	0.04
217967	_s_at	4H	0.04
205069	_s_at	4H	0.04
217947	_at	4H	0.04
203538	_at	4H	0.04
1554300	_a_at	4H	0.04
223439	_at	4H	0.04
201059	_at	4H	0.04
216021	_s_at	4H	0.04
1552503	_at	4H	0.04
226168	_at	4H	0.04
218286	_s_at	4H	0.04
218191	_s_at	4H	0.04
1555772	_a_at	4H	0.04
1320	_at	4H	0.04
200007	_at	4H	0.04
204416	_x_at	4H	0.04
224099	_at	4H	0.04
1554614	_a_at	4H	0.04
205506	_at	4H	0.04
213751	_at	4H	0.04
223754	_at	4H	0.04
207583	_at	4H	0.04
226642	_s_at	4H	0.04
207021	_at	4H	0.04
204243	_at	4H	0.04
218604	_at	4H	0.04
231768	_at	4H	0.04
217216	_x_at	4H	0.04
226656	_at	4H	0.04
206025	_s_at	4H	0.04
227999	_at	4H	0.04
205469	_s_at	4H	0.04
207010	_at	4H	0.04
208506	_at	4H	0.04
1552788	_a_at	4H	0.04
203799	_at	4H	0.04
210335	_at	4H	0.04
207798	_s_at	4H	0.04
207387	_s_at	4H	0.04
201061	_s_at	4H	0.04
243477	_at	4H	0.04
222143	_s_at	4H	0.04
214732	_at	4H	0.04
216202	_s_at	4H	0.04
1554690	_a_at	4H	0.04
242584	_at	4H	0.04
208577	_at	4H	0.04

207924	_x_at	4H	0.04
211505	_s_at	4H	0.04
205760	_s_at	4H	0.04
209515	_s_at	4H	0.04
218527	_at	4H	0.04
215667	_x_at	4H	0.04
230887	_at	4H	0.04
200977	_s_at	4H	0.04
208086	_s_at	4H	0.04
218573	_at	4H	0.04
209784	_s_at	4H	0.04
206136	_at	4H	0.04
204540	_at	4H	0.04
218094	_s_at	4H	0.04
204487	_s_at	4H	0.04
212464	_s_at	4H	0.04
204944	_at	4H	0.04
204020	_at	4H	0.04
214574	_x_at	4H	0.04
218811	_at	4H	0.04
205006	_s_at	4H	0.04
208454	_s_at	4H	0.04
220901	_at	4H	0.04
200934	_at	4H	0.04
204838	_s_at	4H	0.04
219966	_x_at	4H	0.04
201868	_s_at	4H	0.04
201358	_s_at	4H	0.04
213865	_at	4H	0.04
231270	_at	4H	0.04
207738	_s_at	4H	0.04
201665	_x_at	4H	0.04
206860	_s_at	4H	0.04
211940	_x_at	4H	0.04
228844	_at	4H	0.04
217167	_x_at	4H	0.04
207829	_s_at	4H	0.04
203893	_at	4H	0.04
219220	_x_at	4H	0.04
233751	_at	4H	0.04
213414	_s_at	4H	0.04
212905	_at	4H	0.04
213523	_at	4H	0.04
202488	_s_at	4H	0.04
206629	v t	4H	0.04
209150	_s_at	4H	0.04
218641	_at	4H	0.04
222512	v t	4H	0.04
235114	_x_at	4H	0.04
216388	_s_at	4H	0.04
220797	_at	4H	0.04
209467	_s_at	4H	0.04
207196	_s_at	4H	0.04
206604	_at	4H	0.04
211911	_x_at	4H	0.04
224131	_at	4H	0.04
208782	_at	4H	0.04
205223	_at	4H	0.04
204169	_at	4H	0.04
219516	_at	4H	0.04
226121	_at	4H	0.04
204069	_at	4H	0.04
206970	_at	4H	0.04
226259	_at	4H	0.04

21398_s_at 4H 0.04
 201604_s_at 4H 0.04
 223129_x_at 4H 0.04
 206735_at 4H 0.04
 220597_s_at 4H 0.04
 224450_s_at 4H 0.04
 223289_s_at 4H 0.04
 228050_at 4H 0.04
 212359_s_at 4H 0.04
 204127_at 4H 0.04
 1552486_s_at 4H 0.04
 214847_s_at 4H 0.04
 218614_at 4H 0.04
 207659_s_at 4H 0.04
 218745_x_at 4H 0.04
 235250_at 4H 0.04
 203194_s_at 4H 0.04
 213519_s_at 4H 0.04
 221449_s_at 4H 0.04
 227695_at 4H 0.04
 221161_at 4H 0.04
 212204_at 4H 0.04
 206417_at 4H 0.04
 203725_at 4H 0.04
 212265_at 4H 0.04
 200847_s_at 4H 0.04
 218283_at 4H 0.04
 223053_x_at 4H 0.04
 203667_at 4H 0.04
 203828_s_at 4H 0.04
 218022_at 4H 0.04
 224779_s_at 4H 0.04
 160020_at 4H 0.04
 211531_x_at 4H 0.04
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 222676_at 4H 0.04
 1569385_s_at 4H 0.04
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 219817_at 4H 0.04
 217881_s_at 4H 0.04
 221422_s_at 4H 0.04
 201840_at 4H 0.04
 212888_at 4H 0.04
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207798	_s_at	4L	4.548173e-03
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 224669 _at 4V 9.540365e-03
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 1552863 _a_at 4V 9.60222e-03
 222739 _at 4V 9.602496e-03
 226952 _at 4V 9.614726e-03
 226119 _at 4V 9.614726e-03
 226050 _at 4V 9.614726e-03
 223944 _at 4V 9.614726e-03
 226361 _at 4V 9.614726e-03
 226970 _at 4V 9.614726e-03
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 226704 _at 4V 9.614726e-03
 225610 _at 4V 9.614726e-03
 229250 _at 4V 9.614726e-03
 242041 _at 4V 9.614726e-03
 229285 _at 4V 9.614726e-03
 230983 _at 4V 9.614726e-03
 242584 _at 4V 9.614726e-03
 239212 _at 4V 9.614726e-03
 235057 _at 4V 9.614726e-03
 219435 _at 4V 9.614726e-03

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223380_s_at	4V	9.614726e-03	201046_s_at	4V	9.783055e-03
222753_s_at	4V	9.614726e-03	230323_s_at	4V	9.786148 β -03
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202544_at	4V	9.614726e-03	209222_s_at	4V	9.934255e-03
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203126_at	4V	9.614726e-03	228790_at	4V	9.982768e-03
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 217765 _at 4V 0.03
 2164 _at 4V 0.03
 201463 _s_at 4V 0.03
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 211749 _s_at 4V 0.03
 202341 _s_at 4V 0.03
 212790 _x_at 4V 0.03
 220803 _at 4V 0.03
 200082 _s_at 4V 0.03

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234093 _at 4V 0.03
 218511 _s_at 4V 0.03
 200823 _x_at 4V 0.03
 203298 _s_at 4V 0.03
 209248 _at 4V 0.03
 223831 _x_at 4V 0.03
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 220621 _at 4V 0.03
 227240 _at 4V 0.03
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 207927 _at 4V 0.03
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 223151 _at 4V 0.03
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 52164 _at 4V 0.03
 212674 _s_at 4V 0.03
 203391 _at 4V 0.03
 204178 _s_at 4V 0.03
 1553863 _at 4V 0.03
 209440 _at 4V 0.03
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 220778 _x_at 4V 0.03
 211964 _at 4V 0.03
 202483 _s_at 4V 0.03
 223256 _at 4V 0.03
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 211092 _s_at 4V 0.03
 212886 _at 4V 0.03
 208042 _at 4V 0.03
 243456 _at 4V 0.03
 1553770 _a_at 4V 0.04
 226984 _at 4V 0.04
 1553087 _at 4V 0.04
 203597 _s_at 4V 0.04
 223706 _at 4V 0.04
 222678 _s_at 4V 0.04

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200959	_at	4V	0.04							
218603	_at	4V	0.04							
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240603	s_at	4V	0.04							
223011	s_at	4V	0.04							
203679	_at	4V	0.04							
202635	s_at	4V	0.04							
205051	s_at	4V	0.04							
203698	s_at	4V	0.04							
48808	_at	4V	0.04							
201966	_at	4V	0.04							
219762	s_at	4V	0.04							
219333	s_at	4V	0.04							
1553652	a_at	4V	0.04							
203672	x_at	4V	0.04							
209962	_at	4V	0.04							
224395	s_at	4V	0.04							
204278	s_at	4V	0.04							
204499	_at	4V	0.04							
209211	_at	4V	0.04							
1553993	s_at	4V	0.04							
229770	_at	4V	0.04							
226319	s_at	4V	0.04							
219933	_at	4V	0.04							
213160	_at	4V	0.04							
213492	_at	4V	0.04							
210341	_at	4V	0.04							
208827	_at	4V	0.04							
200818	_at	4V	0.04							
226036	x_at	4V	0.04							
224093	_at	4V	0.04							
219329	s_at	4V	0.04							
204602	_at	4V	0.04							
233408	_at	4V	0.04							
204297	_at	4V	0.04							
206864	s_at	4V	0.04							
227063	_at	4V	0.04							
240626	_at	4V	0.04							
1554085	_at	4V	0.04							
208061	_at	4V	0.04							
224683	_at	4V	0.04							
235258	_at	4V	0.04							
213043	s_at	4V	0.04							
225032	_at	4V	0.04							
204075	s_at	4V	0.04							
232843	s_at	4V	0.04							
225253	s_at	4V	0.04							
210303	_at	4V	0.04							
1561429	a_at	4V	0.04							
200864	s_at	4V	0.04							
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224206	x_at	4V	0.04							
1562433	_at	4V	0.04							
223346	_at	4V	0.04							
214652	_at	4V	0.04							
216021	s_at	4V	0.04							
1552503	_at	4V	0.04							
202033	s_at	4V	0.04							
1555772	a_at	4V	0.04							
1320	_at	4V	0.04							
200007	_at	4V	0.04							

226214	_at	4V	0.04
208442	s_at	4V	0.04
204416	x_at	4V	0.04
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218722	s_at	4V	0.04
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205506	_at	4V	0.04
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219457	s_at	4V	0.04
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217216	x_at	4V	0.04
219266	_at	4V	0.04
205382	s_at	4V	0.04
207010	_at	4V	0.04
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221326	s_at	4V	0.04
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207924	x_at	4V	0.04
217846	_at	4V	0.04
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230887	_at	4V	0.04
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215493	x_at	4V	0.04
218811	_at	4V	0.04
205006	s_at	4V	0.04
38037	_at	4V	0.04
212473	s_at	4V	0.04
222295	x_at	4V	0.04
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212348	s_at	4V	0.04
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"	1555298	_a_at	4V	0.04
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	212638	_s_at	4V	0.04
	228736	_at	4V	0.04
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	204186	_s_at	4V	0.04
	211842	_s_at	4V	0.04
	220421	_at	4V	0.04
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	200712	_s_at	4V	0.04
	238001	_at	4V	0.04
	219608	_s_at	4V	0.04
	215718	_s_at	4V	0.04
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	210617	_at	4V	0.04
	204461	_x_at	4V	0.04
	200701	_at	4V	0.04
	231778	_at	4V	0.04
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	1555814	_a_at	4V	0.04
	201433	_s_at	4V	0.04
	201914	_s_at	4V	0.04
	228703	_at	4V	0.04
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	238516	_at	4V	0.04
	204771	_s_at	4V	0.04
	221670	_s_at	4V	0.04
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	201186	_at	4V	0.04
	212966	_at	4V	0.04
	207619	_at	4V	0.04

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200601_at	5G	9.414546e-03
217819_at	5G	9.417991e-03
202565_s_at	5G	9.436678e-03
202572_s_at	5G	9.484365e-03
211471_s_at	5G	9.515525e-03
220467_at	5G	9.515572e-03
219627_at	5G	9.53257e-03
203799_at	5G	9.535298e-03
227236_at	5G	9.53837e-03
218753_at	5G	9.541061e-03
223047_at	5G	9.546636e-03
217902_s_at	5G	9.594088e-03
217762_s_at	5G	9.594407e-03
231809_x_at	5G	9.599765e-03
203544_s_at	5G	9.621376e-03
218060_s_at	5G	9.639582e-03
206861_s_at	5G	9.639586e-03
223886_s_at	5G	9.64344e-03
210129_s_at	5G	9.685817e-03
210142_x_at	5G	9.695538e-03
227268_at	5G	9.758064e-03
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202241_at	5G	9.77037e-03
205213_at	5G	9.780573e-03
212625_at	5G	9.811029e-03
204811_s_at	5G	9.831761e-03
217989_at	5G	9.855033e-03
213206_at	5G	9.875773e-03

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206765_at	5G	9.937505e-03
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205180_s_at	5G	9.942766e-03
219581_at	5G	9.949526e-03
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"22'Mi O₂ at 5H 4.204851e-03

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 227143 _s_at 5H 4.204851e-03
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 226957 _x_at 5H 4.204851e-03
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 236259 _at 5H 4.204851e-03
 38964 _r_at 5H 4.204851e-03
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 38892 _at 5H 4.204851e-03
 228648 _at 5H 4.204851e-03
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 243 _g_at 5H 4.204851e-03
 35160 _at 5H 4.204851e-03
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 235359 _at 5H 4.204851e-03
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 55093 _at 5H 4.204851e-03
 55705 _at 5H 4.204851e-03

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hum _alu_at 5H 4.204851e-03
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 218782 _s_at 5H 4.204851e-03
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 213348 _at 5H 4.204851e-03
 214730 _s_at 5H 4.204851e-03
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 217751 _at 5H 4.204851e-03
 218463 _s_at 5H 4.204851e-03
 218357 _s_at 5H 4.204851e-03
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 217984 _at 5H 4.204851e-03
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 216231 _s_at 5H 4.204851e-03
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 217923 _at 5H 4.204851e-03
 215089 _s_at 5H 4.204851e-03
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 215737 _x_at 5H 4.204851e-03

218099 _at 5H 4.204851e-03

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203816 _at 5H 4.204851e-03

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203514 _at 5H 4.204851e-03

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203262 _s_at 5H 4.204851e-03

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204691 _x_at 5H 4.204851e-03

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203648 _at 5H 4.204851e-03

203320 _at 5H 4.204851e-03

203668 _at 5H 4.204851e-03

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204646 _at 5H 4.204851e-03

203311 _s_at 5H 4.204851e-03

203317 _at 5H 4.204851e-03

203286 _at 5H 4.204851e-03

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203359 _s_at 5H 4.204851e-03

204214 _s_at 5H 4.204851e-03

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204524 _at 5H 4.204851e-03

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221214 _s_at 5H 4.204851e-03

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222984 _[at 5H 4.204851e-03

222218 _s_at 5H 4.204851e-03

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222406 _s_at 5H 4.204851e-03

222396 _at 5H 4.204851e-03

223064 _at 5H 4.204851e-03

222524 _s_at 5H 4.204851e-03

221849 _s_at 5H 4.204851e-03

222826 _at 5H 4.204851e-03

223145 _s_at 5H 4.204851e-03

223066 _at 5H 4.204851e-03

223398 _at 5H 4.204851e-03

223070 [at 5H 4.204851e-03

221269 _s_at 5H 4.204851e-03

223000 _s_at 5H 4.204851e-03

221190 _s_at 5H 4.204851e-03

222985 _at 5H 4.204851e-03

223034 _s_at 5H 4.204851e-03

220615 _s_at 5H 4.204851e-03

211750 _x_at 5H 4.204851e-03

211025 _x_at 5H 4.204851e-03

210418 _s_at 5H 4.204851e-03

211752 _s_at 5H 4.204851e-03

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211999 _at 5H 4.204851e-03

211745 _x_at 5H 4.204851e-03

211576 _s_at 5H 4.204851e-03

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211609 _x_at 5H 4.204851e-03

209906 _at 5H 4.204851e-03

211289 _x_at 5H 4.204851e-03

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209880 _s_at 5H 4.204851e-03

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202197 _at 5H 4.204851e-03

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202497 _x_at 5H 4.204851e-03

202860 _at 5H 4.204851e-03

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202428 _x_at 5H 4.204851e-03

202876 _s_at 5H 4.204851e-03

202386 _s_at 5H 4.204851e-03

202487 _s_at 5H 4.204851e-03

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202426 _s_at 5H 4.204851e-03

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202360 _at 5H 4.204851e-03

202716 _at 5H 4.204851e-03

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202024 _at 5H 4.204851e-03

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204928 _s_at 5H 4.204851e-03

206968 _s_at 5H 4.204851e-03

205898 _at 5H 4.204851e-03

205812 _s_at 5H 4.204851e-03

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201798	_s_at	5H	4.204851e-03
201441	_at	5H	4.204851e-03
200971	_s_at	5H	4.204851e-03
201795	_at	5H	4.204851e-03
201223	_s_at	5H	4.204851e-03
201320	_at	5H	4.204851e-03
201858	_s_at	5H	4.204851e-03
201151	_s_at	5H	4.204851e-03
201050	_at	5H	4.204851e-03
201255	_x_at	5H	4.204851e-03
201597	_at	5H	4.204851e-03
201095	_at	5H	4.204851e-03
201114	_x_at	5H	4.204851e-03
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201814	_at	5H	4.204851e-03
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201587	_s_at	5H	4.204851e-03
201805	_at	5H	4.204851e-03
201166	_s_at	5H	4.204851e-03
201358	_s_at	5H	4.204851e-03
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201863	_at	5H	4.204851e-03
201576	_s_at	5H	4.204851e-03
201175	_at	5H	4.204851e-03
201032	_at	5H	4.204851e-03
201598	_s_at	5H	4.204851e-03
201594	_s_at	5H	4.204851e-03
201915	_at	5H	4.204851e-03
201720	_s_at	5H	4.204851e-03
200857	_s_at	5H	4.204851e-03
201439	_at	5H	4.204851e-03
201818	_at	5H	4.204851e-03
201336	_at	5H	4.204851e-03
201807	_at	5H	4.204851e-03
201040	_at	5H	4.204851e-03
200950	_at	5H	4.204851e-03
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221704	_s_at	5H	4.342468e-03
223199	_at	5H	4.401465e-03
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216457	_s_at	5H	4.584445e-03
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207075	_at	5H	4.739743e-03
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201895	_at	5H	4.755023e-03
201400	_at	5H	4.75618e-03
235744	_at	5H	4.766764e-03
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 200654 _at 5H 8.918234e-03
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218865_at	5J	0.04	228774_at	5K	0.01
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217716_s_at	5K	1.926726e-03	226379_s_at	5K	0.01
244509_at	5K	2.194146e-03	217529_at	5K	0.01
1569887_a_at	5K	2.858068e-03	204572_s_at	5K	0.01
214775_at	5K	2.895957e-03	209853_s_at	5K	0.01

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218433_at	5K	0.01				
205548_s_at	5K	0.01				
201868_s_at	5K	0.01				
227195_at	5K	0.01				
219624_at	5K	0.01				
217893_s_at	5K	0.01				
211714_x_at	5K	0.01				
229251_s_at	5K	0.01				
219931_s_at	5K	0.01				
223511_at	5K	0.01				
209198_s_at	5K	0.01				
220777_at	5K	0.01				
1555775_a_at	5K	0.01				
217721_at	5K	0.01				
203442_x_at	5K	0.01				
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229862_x_at	5K	0.01				
228556_at	5K	0.01				
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213849_s_at	5K	0.02				
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224768_at	5K	0.02				
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210200_at	5K	0.02				
223658_at	5K	0.02				
223485_at	5K	0.02				
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²⁷⁶ ²⁷⁷ ²⁷⁸ ²⁷⁹ ²⁸⁰ ²⁸¹ ²⁸² ²⁸³ ²⁸⁴ ²⁸⁵ ²⁸⁶ ²⁸⁷ ²⁸⁸ ²⁸⁹ ²⁹⁰ ²⁹¹ ²⁹² ²⁹³ ²⁹⁴ ²⁹⁵ ²⁹⁶ ²⁹⁷ ²⁹⁸ ²⁹⁹ ³⁰⁰ ³⁰¹ ³⁰² ³⁰³ ³⁰⁴ ³⁰⁵ ³⁰⁶ ³⁰⁷ ³⁰⁸ ³⁰⁹ ³¹⁰ ³¹¹ ³¹² ³¹³ ³¹⁴ ³¹⁵ ³¹⁶ ³¹⁷ ³¹⁸ ³¹⁹ ³²⁰ ³²¹ ³²² ³²³ ³²⁴ ³²⁵ ³²⁶ ³²⁷ ³²⁸ ³²⁹ ³³⁰ ³³¹ ³³² ³³³ ³³⁴ ³³⁵ ³³⁶ ³³⁷ ³³⁸ ³³⁹ ³⁴⁰ ³⁴¹ ³⁴² ³⁴³ ³⁴⁴ ³⁴⁵ ³⁴⁶ ³⁴⁷ ³⁴⁸ ³⁴⁹ ³⁵⁰ ³⁵¹ ³⁵² ³⁵³ ³⁵⁴ ³⁵⁵ ³⁵⁶ ³⁵⁷ ³⁵⁸ ³⁵⁹ ³⁶⁰ ³⁶¹ ³⁶² ³⁶³ ³⁶⁴ ³⁶⁵ ³⁶⁶ ³⁶⁷ ³⁶⁸ ³⁶⁹ ³⁷⁰ ³⁷¹ ³⁷² ³⁷³ ³⁷⁴ ³⁷⁵ ³⁷⁶ ³⁷⁷ ³⁷⁸ ³⁷⁹ ³⁸⁰ ³⁸¹ ³⁸² ³⁸³ ³⁸⁴ ³⁸⁵ ³⁸⁶ ³⁸⁷ ³⁸⁸ ³⁸⁹ ³⁹⁰ ³⁹¹ ³⁹² ³⁹³ ³⁹⁴ ³⁹⁵ ³⁹⁶ ³⁹⁷ ³⁹⁸ ³⁹⁹ ⁴⁰⁰ ⁴⁰¹ ⁴⁰² ⁴⁰³ ⁴⁰⁴ ⁴⁰⁵ ⁴⁰⁶ ⁴⁰⁷ ⁴⁰⁸ ⁴⁰⁹ ⁴¹⁰ ⁴¹¹ ⁴¹² ⁴¹³ ⁴¹⁴ ⁴¹⁵ ⁴¹⁶ ⁴¹⁷ ⁴¹⁸ ⁴¹⁹ ⁴²⁰ ⁴²¹ ⁴²² ⁴²³ ⁴²⁴ ⁴²⁵ ⁴²⁶ ⁴²⁷ ⁴²⁸ ⁴²⁹ ⁴³⁰ ⁴³¹ ⁴³² ⁴³³ ⁴³⁴ ⁴³⁵ ⁴³⁶ ⁴³⁷ ⁴³⁸ ⁴³⁹ ⁴⁴⁰ ⁴⁴¹ ⁴⁴² ⁴⁴³ ⁴⁴⁴ ⁴⁴⁵ ⁴⁴⁶ ⁴⁴⁷ ⁴⁴⁸ ⁴⁴⁹ ⁴⁵⁰ ⁴⁵¹ ⁴⁵² ⁴⁵³ ⁴⁵⁴ ⁴⁵⁵ ⁴⁵⁶ ⁴⁵⁷ ⁴⁵⁸ ⁴⁵⁹ ⁴⁶⁰ ⁴⁶¹ ⁴⁶² ⁴⁶³ ⁴⁶⁴ ⁴⁶⁵ ⁴⁶⁶ ⁴⁶⁷ ⁴⁶⁸ ⁴⁶⁹ ⁴⁷⁰ ⁴⁷¹ ⁴⁷² ⁴⁷³ ⁴⁷⁴ ⁴⁷⁵ ⁴⁷⁶ ⁴⁷⁷ ⁴⁷⁸ ⁴⁷⁹ ⁴⁸⁰ ⁴⁸¹ ⁴⁸² ⁴⁸³ ⁴⁸⁴ ⁴⁸⁵ ⁴⁸⁶ ⁴⁸⁷ ⁴⁸⁸ ⁴⁸⁹ ⁴⁹⁰ ⁴⁹¹ ⁴⁹² ⁴⁹³ ⁴⁹⁴ ⁴⁹⁵ ⁴⁹⁶ ⁴⁹⁷ ⁴⁹⁸ ⁴⁹⁹ ⁵⁰⁰ ⁵⁰¹ ⁵⁰² ⁵⁰³ ⁵⁰⁴ ⁵⁰⁵ ⁵⁰⁶ ⁵⁰⁷ ⁵⁰⁸ ⁵⁰⁹ ⁵¹⁰ ⁵¹¹ ⁵¹² ⁵¹³ ⁵¹⁴ ⁵¹⁵ ⁵¹⁶ ⁵¹⁷ ⁵¹⁸ ⁵¹⁹ ⁵²⁰ ⁵²¹ ⁵²² ⁵²³ ⁵²⁴ ⁵²⁵ 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 200632 _s_at 5N 0.01
 65588 _at 5N 0.01
 204143 _s_at 5N 0.01
 239067 _s_at 5N 0.01

! " # \$ % & ' () * + , - . / : ; < = > ? @ A B C D E F G H I J K L M N O P Q R S T U V W X Y Z [\] ^ _ ` a b c d e f g h i j k l m n o p q r s t u v w x y z { | } ~ ¡ ¢ £ ¤ ¥ ¦ § ¨ © ª « ¬ ® ¯ ° ± ² ³ ´ µ ¶ · ¸ ¹ º » ¼ ½ ¾ ¿

202425	_x_at	5N	0.01	231252__at	5N	0.02
213314	_at	5N	0.01	220255__at	5N	0.02
206687	_s_at	5N	0.01	202362__at	5N	0.02
221600	_s_at	5N	0.01	208658__at	5N	0.02
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238365	_s_at	5N	0.01	221875_x_at	5N	0.02
206500	_s_at	5N	0.01	202183_s_at	5N	0.02
AFFX-HUMGAPDH/				205600_x_at	5N	0.02
M33197		5N	0.01	206478_at	5N	0.02
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				204174__at	5N	0.02

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202425		x at	5Q	7.454765e-03
224465		s at	5Q	7.461587e-03
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223318		s at	5Q	7.477942e-03
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1555950	_a_at	5R	0.03		
208967	_s_at	5R	0.03		
205245	_at	5R	0.04		
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227598	_at	5R	0.04		
223217	_s_at	5R	0.04		
205248	_at	5R	0.04		
205692	_s_at	5R	0.04		
202518	_at	5R	0.04		
204398	_s_at	5R	0.04		
1558791	_at	5R	0.04		
214326	_x_at	5R	0.04		
224393	_s_at	5R	0.04		
226611	_s_at	5R	0.04		
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218833	_at	5R	0.04		
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224680_at	5T	0.02		
207113_s_at	5T	0.02		
224345_x_at	5T	0.02		
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224578_at	5T	0.02		
209892_at	5T	0.02		
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204269_at	5T	0.02		
226753_at	5T	0.02		
225850_at	5T	0.02		
208763_s_at	5T	0.02		
225252_at	5T	0.02		
204172_at	5T	0.02		
218319_at	5T	0.02		
203927_at	5T	0.02		
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225997_at	5T	0.02		
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228009_x_at	5T	0.02		
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223176_at	5T	0.02		
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229553_at	5T	0.02		
224904_at	5T	0.02		
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229632_s_at	5T	0.02		
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227447_at	5T	0.02		
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203176_s_at	5T	0.02		
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220521	_s_at	5U	0.03	
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218640	_s_at	5U	0.03	
219635	_at	5U	0.03	
219151	_s_at	5U	0.03	
223158	_s_at	5U	0.03	
204420	_at	5U	0.03	
217764	_s_at	5U	0.03	
212318	_at	5U	0.03	
204065	_at	5U	0.03	
212055	_at	5U	0.03	
205967	_at	5U	0.03	
219507	_at	5U	0.03	
224480	_s_at	5U	0.03	
206792	_x_at	5U	0.03	
221192	_x_at	5U	0.03	
222258	_s_at	5U	0.03	
212629	_s_at	5U	0.03	
202577	_s_at	5U	0.03	
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203542	_s_at	5U	0.03	
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221670	_s_at	5U	0.03	
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214561	_at	5U	0.04	
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209536	_s_at	5U	0.04	
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203370	_s_at	50	0.04	
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205061	_s_at	50	0.04
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224836	_at	50	0.04
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237107	_at	50	0.04
212484	_at	50	0.04
200779	_at	50	0.04
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217299	_s_at	50	0.04
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208248	_x_at	5V	2.91e-05
211696	_x_at	5V	3.01e-05
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217232_x_at	sv	1.09e-04
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200072_s_at	sv	1.17e-04
200677_at	sv	1.17e-04
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201968_s_at	sv	1.23e-04
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209116_x_at	sv	1.31e-04
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203721_s_at	sv	1.35e-04
204018_x_at	sv	1.37e-04
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202797_at	5V	0.01			
219330_at	5V	0.01			
201239_s_at	5V	0.01			
202738_s_at	5V	0.01			
215633_x_at	5V	0.01			
215823_x_at	5V	0.01			
212124_at	5V	0.01			
204490_s_at	5V	0.01			
202513_s_at	5V	0.01			
217388_s_at	5V	0.01			
206015_s_at	5V	0.01			
208945_s_at	5V	0.01			
201575_at	5V	0.01			
218195_at	5V	0.01			
209107_x_at	5V	0.01			
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201786_s_at	5V	0.01			
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203663_s_at	5V	0.01			
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209004_s_at	5V	0.01			

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210097_s_at	5V	0.01
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218242_s_at	5V	0.01
91816_f_at	5V	0.01
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201339_s_at	5V	0.01
217738_at	5V	0.01
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205173_x_at	5V	0.01
201924_at	5V	0.01
211716_x_at	5V	0.01
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217725_x_at	5V	0.01
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219666_at	5V	0.01
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221500_s_at	5V	0.01
208720_s_at	5V	0.01
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AFFX-HUMGAPDH/		
M33197	5V	0.02
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203291_at	5V	0.02

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202232	_s_at	5V	0.02	208578	_at	5V	0.02
212616	_at	5V	0.02	210574	_s_at	5V	0.02
202084	_s_at	5V	0.02	216071	_x_at	5V	0.02
203126	_at	5V	0.02	212265	_at	5V	0.02
213225	_at	5V	0.02	205269	_at	5V	0.02
204632	_at	5V	0.02	211503	_s_at	5V	0.02
209827	_s_at	5V	0.02	204360	_s_at	5V	0.02
209033	_s_at	5V	0.02	210784	_x_at	5V	0.02
221476	_s_at	5V	0.02	217398	_x_at	5V	0.02
212895	_s_at	5V	0.02	221058	_s_at	5V	0.02
209020	_at	5V	0.02	208692	_at	5V	0.02
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201941	_at	5V	0.02	203611	_at	5V	0.02
217986	_s_at	5V	0.02	206875	_s_at	5V	0.02
203614	_at	5V	0.02	220755	_s_at	5V	0.02
202076	_at	5V	0.02	221471	_at	5V	0.02
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202957	_JLt	5V	0.02	211758	_x_at	5V	0.02
207522	_s_at	5V	0.02	211711	_s_at	5V	0.02
203537	_at	5V	0.02	201369	_s_at	5V	0.02
211101	_x_at	5V	0.02	201493	_s_at	5V	0.02
217028	_at	5V	0.02	208675	_s_at	5V	0.02
204034	_at	5V	0.02	218809	_at	5V	0.02
201352	_at	5V	0.02	220615	_s_at	5V	0.02
214709	_s_at	5V	0.02	211714	_x_at	5V	0.02
207563	_s_at	5V	0.02	210254	_at	5V	0.02
202910	_s_at	5V	0.02	215157	_x_at	5V	0.02
207408	_at	5V	0.02	201740	_at	5V	0.02
210579	_s_at	5V	0.02	218048	_at	5V	0.02
202230	_s_at	5V	0.02	202727	_s_at	5V	0.02
200781	_s_at	5V	0.02	212804	_s_at	5V	0.02
204971	_at	5V	0.02	201029	_s_at	5V	0.02
218950	_at	5V	0.02	214264	_s_at	5V	0.02
63009	_at	5V	0.02	201993	_x_at	5V	0.02
215088	_s_at	5V	0.02	213065	_at	5V	0.02
205849	_s_at	5V	0.02	201476	_s_at	5V	0.02
202604	_x_at	5V	0.02	201455	_s_at	5V	0.02
213557	_at	5V	0.02	202103	_at	5V	0.02
218091	_at	5V	0.02	201348	_at	5V	0.02
216252	_x_at	5V	0.02	212188	_at	5V	0.02
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222024	_s_at	5V	0.02	202423	_at	5V	0.02
218520	_at	5V	0.02	221078	_s_at	5V	0.02
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218196	_at	5V	0.02	202534	_x_at	5V	0.02
218870	_at	5V	0.02	221479	_s_at	5V	0.02
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202817	_s_at	5V	0.02	204050	_s_at	5V	0.02
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201409	s_at	5V	0.02
202736	s_at	5V	0.02
203142	s_at	5V	0.02
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213940	s_at	5V	0.02
202824	s_at	5V	0.02
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218322	s_at	5V	0.02
217941	s_at	5V	0.02
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211069	s_at	5V	0.02
202364	at	5V	0.02
201699	at	5V	0.02
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222035	s_at	5V	0.02
221483	s_at	5V	0.02
201670	s_at	5V	0.02
209928	s_at	5V	0.02
200033	at	5V	0.02
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218987	at	5V	0.02
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203487	s_at	5V	0.02
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220023	at	5V	0.03
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206576	s_at	5V	0.03
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202221	s_at	5V	0.03
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220036	s_at	5V	0.03

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213061	s_at	5V	0.03
218936	s_at	5V	0.03
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212476	at	5V	0.03
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200928	s_at	5V	0.03
205147	x_at	5V	0.03
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201637	s_at	5V	0.03
203360	s_at	5V	0.03
201054	at	5V	0.03
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209494	s_at	5V	0.03
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200825_s_at	5V	0.03
212334_at	5V	0.03
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212594_at	6A	0.02
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205425	_at	6A	0.02						
217878	_s_at	6A	0.02						
235923	_at	6A	0.02						
235409	_at	6A	0.02						
225698	_at	6A	0.02						
214672	_at	6A	0.02						
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206848	_at	6A	0.02						
200640	_at	6A	0.02						
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200605	_s_at	6A	0.02						
33646	_g_at	6A	0.02						
209137	_s_at	6A	0.02						
204501	_at	6A	0.02						
235170	_at	6A	0.02						
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1554628	_at	6A	0.02						
219459	_at	6A	0.02						
221036	_s_at	6A	0.02						
222343	_at	6A	0.02						
209153	_s_at	6A	0.02						
203692	_s_at	6A	0.02						
205259	_at	6A	0.02						
224772	_at	6A	0.02						
220961	_s_at	6A	0.02						
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213969	_x_at	6A	0.02						
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208914	_at	6A	0.02						
207809	_s_at	6A	0.02						
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201078	at	6A	0.03	210250	x_at	6A	0.04
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201346_at	6A	0.04	201517_at	6A	0.04
218259_at	6A	0.04	1553165_at	6A	0.04
213092_x_at	6A	0.04	217977_at	6A	0.04

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223482_at	6A	0.04	214501_s_at	6B	6.805837e-03
210624_s_at	6A	0.04	203922_s_at	6B	6.809059e-03
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227693	_at	7F	0.03
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